Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 8-16 (2020)

Comparative characteristics of bioelectric activity of the brain in long-livers from different regions of the Republic of Azerbaijan

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Accepted for publication: 17 April 2020

With application of modern technique of computerized electroencephalography comparative data of spectral-frequency analysis of baseline electroencephalography of the long-livers having high indexes of longevity and living in southern part of Azerbaijan – in Lenkoran (most of the areas are plains) and Lerik (mountainous) regions – are presented in the article. The undertaken electrophysiological studies revealed resembling and different features in the electroencephalogram (EEG) of the long-livers' populations within the different geographic areas of Azerbaijan. On the basis of comparative analysis of the recordings of baseline EEG of the long-livers of both regions one can reveal protective and inhibitory effects of the sub-cortical structures on the activity of the cortex (coming from prevalence of the indexes of delta- and theta-rhythms) accompanied with their low activities. However, in the long-livers of the Lerik region, in contrast to the long-livers of the Lenkoran region, compensatory upregulation of the activating effect of the mesencephalic reticular formation to the brain cortex was noticed. Along with the general properties of EEG, the differences in EEG patterns indicate different directions of the activation of the brain compensatory mechanisms, which gives grounds to put forward the conjecture saying that in relation to age-related changes, reorganization of neurons' communications in the central nervous system and support of high level of the brain activity in the long-livers require engagement of much more internal resources.

Keywords: *Long-livers, electroencephalogram, spectral analysis, spectral power, index, brain functional state.*

INTRODUCTION

Studies of the long-livers are one of the most actual problems of medical and biological research. The long-livers themselves present convincing illustration of physiological aging and unravelling its mechanisms presents evidences of direct relations of its processes to the changes occurring in the brain and central nervous system (CNS) (Gomazkov, 2012).

Electroencephalographic method, being used in the studies of the brain activity, its age-related changes, at present time is not just the method of analysis of the brain functional state in the clinical practice, but as well is the widely used technique for conducting research in the field of fundamental neurosciences (Kambarova et al., 2010). Bioelectric activity of the brain is closely related to the main functional states: quietness, alertness, sleep, high scale activity (Polunina, 2012). One of the main approaches of the studies of the aging-related changes is formation of a map of baseline bioelectric activity of the brain. Baseline state of electroencephalogram (closed eyes, quiet awake state) differs with its relative stable parameters (McEvoy et al., 2001) and reflects preparedness to the brain's following activity (Klimesch et al., 2006; Razoumnikova, 2003; Volf et al., 2010).

Basing on the brain-specific "language" of bioelectric activity, comparative analysis of the age-related dynamics of functional state of CNS makes it possible to determine aging rates of the brain depending on its adherence to different national-ethnic peculiarities. Particularly, according to the results of comparative electrophysiological studies conducted among the populations of Ukraine, Abkhazia and Azerbaijan (Kuznetsova,

doi.org/10.29228/jlsb.36

2008), the changes noticed in the EEG structures in Ukrainians, are more intensive than those in Abkhazians and Azerbaijanians. Meanwhile, the age-related changes of bioelectric activity in Azerbaijanians are higher relatively to Abkhazians. Having been the country characterized with high level of long-living, in several regions of Azerbaijan high level of the long-living index is observed among the local people that is termed as the group-like or population phenomenon (Kozlov, 1982). One of the zones with high long-living index is the southern zone located at the bottom of the Talysh Mountains. The main objective of the research is comparative study of the EEG structure reflecting the functional state of CNS of the long-livers living in southern zone of Azerbaijan, in Lenkoran (most of the areas are plains), and in Lerik (mountainous) possessing high levels of long-living index.

MATERIALS AND METHODS

The study comprised 36 actually healthy long-livers (at the age of over 90 years old), born and still living in Lerik (19 persons) and Lenkoran (17 persons) regions of Aerbaijan. The persons having in their disease history unconscious states, dreaming and head-brain injuries were excluded from consideration.

The EEG was fixed in 16 standard points unipolar on International System "10-20%" (Fp1/Fp2, F3/F4, C3/C4, P3/P4, O1/O2, F7/F8, T3/T4, T5/T6) with application of computer complex (Kulaichev,2007) "Neuron-Spectre-5" (Russia, 2012) under constant time 0.3 sec. The lane of filtration made 0.5-35 Hz. The frequency of digitalization made 200 Hz. Ipsilateral ear electrode was used as a reference electrode. Registration was undertaken under conditions of relaxed awakeness with closed eyes which is characterized with definite organization of EEG (Marx et al., 2004; Polunina, 2012). Computer processing of EEG was done with application of system analysis on the program of "Neuron-Spectre-Net" (Russia, 2012). Epoch of analysis made 6 sec. Each time 10 artefact-free EEG epochs, registered under baseline state, were analysed. For each outlet point with application of Fourier's quick transformation spectral power, frequency, EEG index for frequency ranges: delta (0.5-4 Hz), theta (4-7 Hz), alpha (8-13 Hz, beta-low-frequency (beta-1; 14-17 Hz), beta-high-frequency (beta-2; 20-35 Hz) were calculated.

To analysing the data the standard statistic sets of "Microsoft Excel-2007" were applied. The validity of differences in the studied groups was determined with application of the method of U-Wilkokson-Mann-Whitney criterion with defining prominence of inter-group differences. The critical level of validity was accepted as 5% ($p<0.05$).

RESULTS AND DISCUSSION

During conducting studies the brain bioelectric activity in the relatively quiet status was registered in the long-livers staying in Lenkoran and Lerik regions of Azerbaijan. Relative quietness status provides preparedness to active functioning of the brain and this is one of the important field of studies of the experimental neurophysiology (Razoumnikova, 2003; Volf et al., 2011).

On the basis of spectral analysis with computer program of EEG, being an index of the brain functional activity of the long-livers of Lenkoran and Lerik regions of Azerbaijan, comparative analysis of such basic parameters as spectral power (μV^2), index (%) and frequency range (Hz) gave an opportunity of getting novel data.

Analysis of spectral power of EEG (Fig.1) shows that in the long-livers living in both studied regions, averaged spectral power of delta-rhythm composed of low-frequency waves demonstrates dominance in the studied brain cortex areas. The highest values of delta-rhythm (25-30 μV^2) were observed in the area of Fp_1/Fp_2. Spectral power of another low-frequency rhythm – theta-rhythm – revealed dominance in the brain areas of Fp_1/Fp_2, F_3/F_4, C_3/C_4, P_3/P_4 of the long-livers of both regions.

Analysis of spectral power of high-frequency rhythms (alpha, beta 1 and beta 2) showed certain differences in the data. Averaged spectral power of alpha-rhythm in the brain parietal (P_3/P_4) and central (C_3/C_4) cortex is high (3.6-4.8 μV^2) relatively to the values of the occipital cortex (O_1/O_2) in the long-livers of both regions. However, average spectral power of alfa-rhythm in the area of C_3/C_4 of the long-livers living in Lenkoran region

is significantly higher ($p<0.05$) in compaison to the long-livers of Lerik region. Software program "Neuron-Spector.Net" presents an opportunity to analyze separately low-frequency beta 1 (14-19 Hz) and high-frequency beta 2 (20-35 Hz) rhythms of considered to be high-frequency beta rhythm. On the basis of this analysis differing results were obtained. Particularly, statistic analysis showed that averaged spectral power of beta1 rhythm in the brain cortical areas of Fp_1/Fp_2, C_4, P_3/ P_4, O_1,T_5/T_6 of the long-livers living in Lenkoran region was significant lower in comparison to the long-livers living in Lerik region ($p<0.05$). Similarly, spectral power of high-frequency beta 2 rhythm in all the studied brain cortex areas in exception of C_3 and T_3 areas in the long-livers of Lenkoran region was significantly lower relatively to the long-livers of Lerik region. High degree of statistic validity was observed in the brain frontal (Fp_1/Fp_2, F_3/F_4; $p<0.001$), parietal (P_3/P_4; $p<0.001$) and posterior temporal (T_5/T_6; $p<0.001$) cortical areas.

While conducting comparative analysis of a mean index (Fig. 2), one of the main parametric indexes of EEG, the prevalence of delta rhythm of the studied cortical areas of the brain of the long-livers, living in both regions, over the other rhythms was revealed. The mean index of this rhythm had the highest values (59-62%) in the anterior frontal (Fp_1/Fp_2) area. The mean index of theta rhythm of the studied cortical areas of the brain of the long-livers of both regions as well dominated over other rhythms and he highest values (22-24%) were noticed in the central (C_3/C_4) and parietal (P_3/P_4) areas. Statistical analysis of mean indexes of high frequency alpha, beta 1 and beta 2 rhythms of the long-livers living in Lenkoran region revealed different data relatively to the long-livers living in Lerik region. Particularly, in the long-livers of Lenkoran region in comparison to the long-livers of Lerik region mean indexes of alpha-rhythm in the left frontal (F_3), central (C_3/C_4) and left middle temporal (T_3) areas are significantly high ($p<0.05$). Conversely, mean index of beta 1-rhythm in the right parietal area (P_4), left and right frontal portion of temporal area (F_7/F_8) and left rear temporal area (T_5) of the brain of the long-livers living in Lenkoran region are significantly lower ($p<0.05$), than in the long-livers of Lerik region. This difference is as well observed in the values of mean index of beta 2 rhythm. Mean index of beta 2 rhythm of the long-livers of Lenkoran region in parietal (P_4), occipital (O_1/O_2) and temporal (F_7/F_8, T_3/T_4 and T_5/T_6) areas of the brain cortex are significantly lower, than in the long-livers of Lerik region. The highest difference ($p<0.01$) is observed in the frontal portions of temporal area (F_7/F_8).

As it issues from the results, the values of mean index of alpha rhythm significantly dominated in the certain cortical areas of the brain of the long-livers of Lenkoran region. Though low values of this index in the long-livers of Lerik region, it is accompanied with compensatory upregulation of mean index of beta rhythm.

Basing on statistical analysis of averaged frequencies, though variations of averaged frequency within range of 0.97-1.5 Hz of delta rhythm in the studied cortical areas of the brain of the long-livers living in both regions, significant differences were not revealed. However, in the long-livers of both regions averaged frequencies of delta rhythm of Fp_1/Fp_2 and O_1 areas were relatively low ($p>0.05$). The frequency of theta rhythm was high in the long-livers of Lenkoran region and made 6.15-6.63 Hz, while in the long-livers of Lerik region it made 5.76-6.28 Hz. However, these changes were not significant. The frequency of alpha rhythm in the long-livers of both regions was observed in the range of 9.33-9.68 Hz.Though the averaged frequency of beta 1 rhythm in different cortical areas in the long-livers of both regions was observed within the range of 16.07-16.64 Hz, prominent differences were not revealed. The analysis of beta 2 rhythm showed certain differences. Basing on the analysis of averaged frequency of beta 2 rhythm in the frontal (Fp_1, F_3), parietal (P_3) and temporal (F_7, T_3) areas of the brain cortex, significant ($p<0.05$) downregulation of its level was noticed.

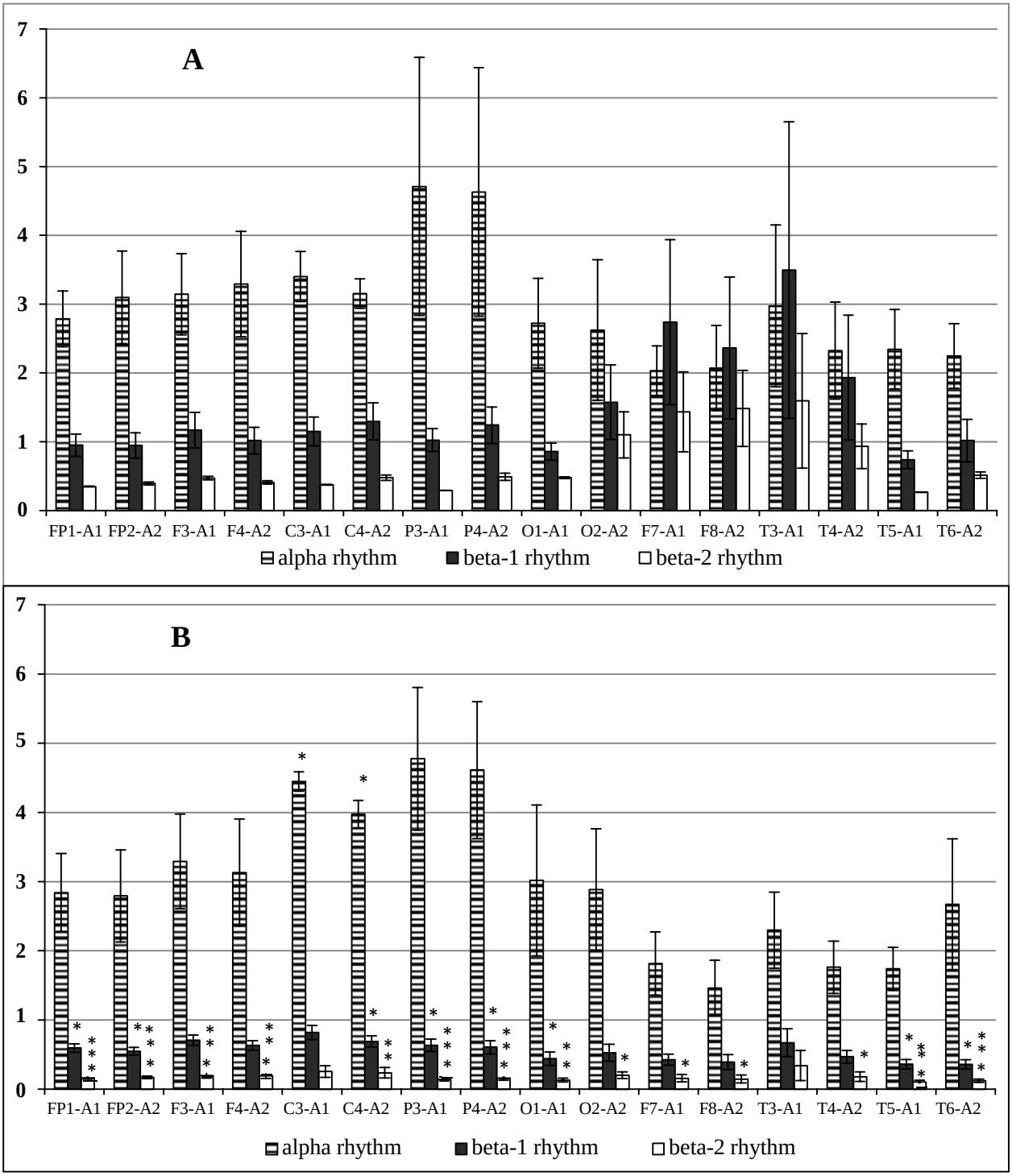

Fig. 1. Background EEG power spectrum from Lerik (A) and Lankaran (B) long-livers.
The horizontal axis represents the labeles of 16 channels, the vertical axis shows power spectrum density (μV^2). * - $p<0.05$, **$p< 0.01$, ***$p< 0.001$

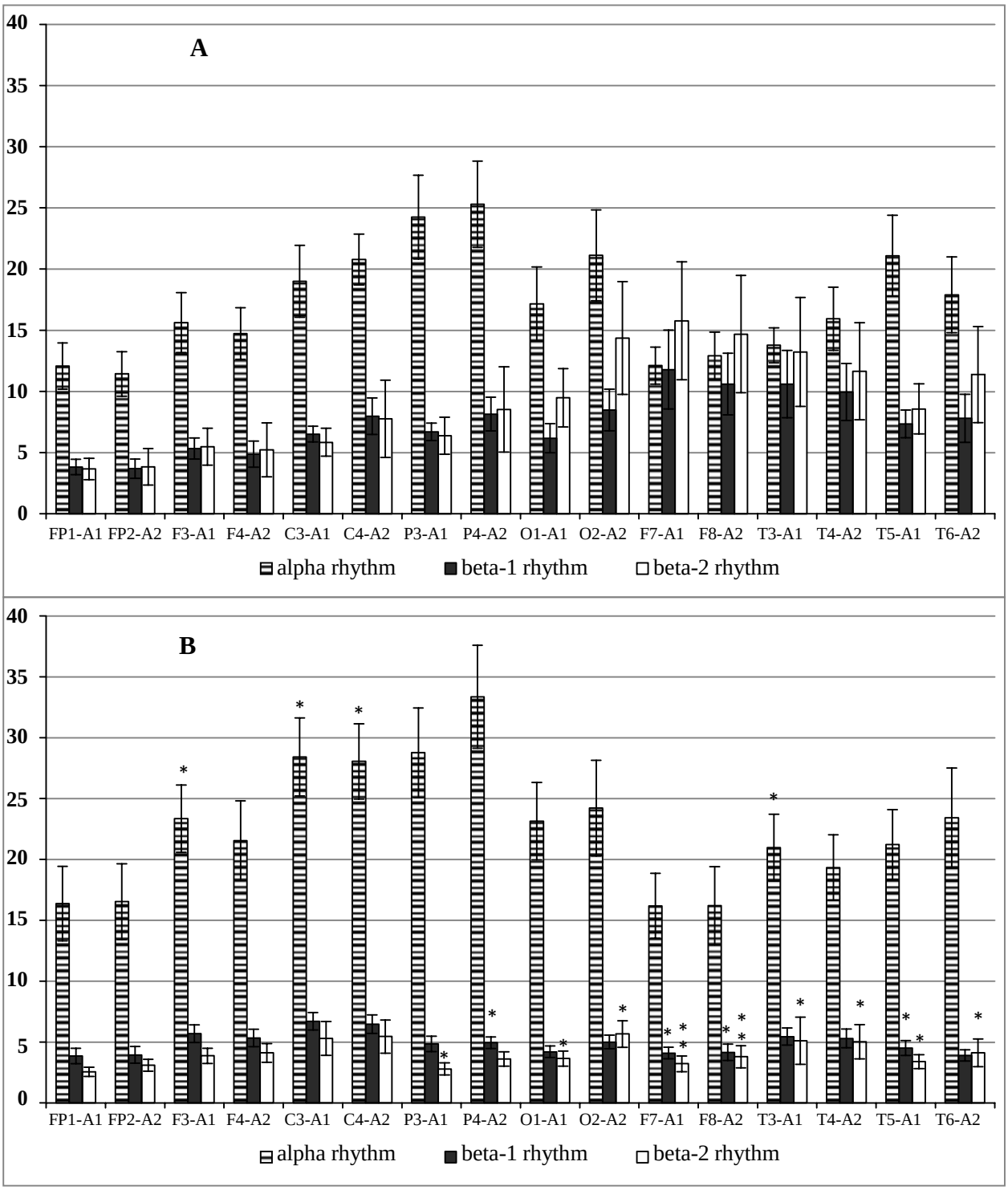

Fig.2. Background EEG rhythm indexes in Lerik (A) and Lankaran (B) long-livers. The horizontal axis shows acronyms of 16 channels, the vertical axis displays rhythms indexes (%). * - $p<0.05$, **$p< 0.01$

As it is seen from the results, EEG analysis of the long-livers, living in Lenkoran and Lerik regions, revealed both similar and distinct features. EEG, being an epoch of neuronal constellations, reflects their changing activity. Due to spectral power of EEG, one can put forward an idea of composition of this epoch. The results of spectral analysis of EEG showed dominance of spectral power and index of slow rhythms (delta and theta rhythms) over other rhythms of the long-livers living in Lenkoran and Lerik regions. In the most of publications concerning age-related changes of slow rhythms (delta and theta rhythms), upregulation of their power, while in the least of cases age-related changes (Blalock et al., 2003; Carlson et al., 2004; Van Cott, 2002) were observed during aging. In total spectral power of EEG, upregulation of relative part of delta rhythm is related to downregulation of the brain functional activity (Deryabina, 2016), while downregulation of frequency of the frontal and occipital cortical areas of the brain relatively to the other areas of the brain cortex is related to downregulation of activating effects of the sub-cortical system which defines the frequency characteristics of slow waves (Moretti et al., 2012). In the publication concerning studies of peculiarities of regional distribution of alpha rhythm (Volf et al., 2011), one of the high frequency rhythms (alpha, beta 1 and beta 2), decline of power of alpha rhythm towards rear portions of brain hemispheres in baseline EEG of the long-livers is explained in terms of more effective functionalization of the brain structures. It is known that rear portions of the brain are engaged in storage of short-term memory and analysis of vision-surrounding images (Klimesch et al., 2006). There are evidences in the literature concerning age-related stability and decline (Liddell et al., 2007) of beta activity. However, the most of publications notice upregulation of high frequency activity (beta rhythm) during normal aging (Volf et al., 2011; Vysata et al., 2012; Zenkov, 2010). There are publications showing positive correlation between upregulation of high frequency waves (beta rhythm) and effectivity of cognitive functions during aging (Volf et al., 2011). Downregulation of power of beta rhythm in the people having primary manifestations of dementia and in the people with psychic impairments (Liddell et al., 2007) supports this idea. The results of our studies showed that in the long-livers living in Lerik region in comparison to the long-livers of Lenkoran region on the background of downregulation of alpha rhythm, compensatory upregulation of spectral power and index of beta rhythm indicates to decline of the activity of thalamo-cortical synchronizational system and prevailing of the activating effects of the mesencephalic reticular formation onto the brain cortex (Gordeev, 2007; Zenkov, 2010). During aging effectivity of brain activity is defined by 2 processes: the level of age-related brain degradation and launching of the compensatory mechanisms (Volf et al., 2011). It should be noted that brain responds to age-related changes through "compensatory support". Studies conducted on the animals, show that adaptation potential of the brain to the age-related changes occurs on account of neurogenesis and changes of synaptic plasticity. These processes are realized through proliferation and differentiation of precursor cells and thereafter redistribution of these nervous cells within the brain structures (Kempermann et al., 2002). The results obtained from the long-livers living in Lerik region showing prevalence of beta activity in frontal and temporal cortical areas of the brain during aging are consistent with the idea of the physiological changes of EEG and upregulation of compensatory activities in these structures.

So, the results of the conducted electrophysiological studies indicate to various changes in EEG and to mobilization of compensatory mechanisms of the brain in different directions in the long-livers, living in different geographic regions of Azerbaijan. Along with age-related development of the involutional changes, these data reflect launching the adaptive-compensatory processes.

CONCLUSIONS

1. Prevalence of indexes of slow rhythms (delta and theta rhythms) in the baseline EEG of the long-livers living in Lenkoran and Lerik regions, indicates to development of protective inhibition and low functional activity of the brain which are related to strengthening of the synchronizing effects of the thalamo-cortical system and decline of the activating effects of the sub-cortical system.

2. Comparative analysis of baseline EEG of the long-livers showed that in the long-livers living in Lerik region, in contrast to the long-livers of Lenkoran region, on the background of decline of alpha rhythm, compensatory upregulation of the spectral power and spectral index of beta rhythm indicates to compensatory strengthening of the activating effects of the mesencephalic reticular formation on the brain cortex.

3. The obtained results of electrophysiological studies show that along with general features, presence of differences in EEG of the long-livers, living in different long-livers populations in different geographic regions of Azerbaijan, indicates to launching of the compensatory mechanisms of the brain in different directions. This give grounds to putting forward an idea that reorganization of the neuronal networks of the central nervous system and supporting active functioning of the brain of the long-livers, subjected to age-related physiological changes, require engagement of much more internal resources.

REFERENCES

Blalock E. M., Chen K. C., Sharrow K *(*2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J. Neuroscience*, **23:** 3807-3819

Carlson C. E., St Louis E. D., Granner M. A. *(*2004) Yield of video EEG monitoring in patients over the age of 50. *Program and abstract of the American Epilepsy Society 58th Annual Meeting*. Abstract **2:** 226 p

Deryabina I.N. (2016) Characteristic of brain electrobiological activity in elderly women with different level of cognitive disorders. *Human Ecology*, **10:** 51-56

Gomazkov O.A. (2012) Cellular and molecular principles of brain aging. *J. Advances in Current Biology*, **132(2):** 141-154.

Gordeev S. A. (2007) Changes in electrical brain activity in patients with panic disorders. *I.P.Pavlov Journal of Higher Nervous Activity*, **57(3):** 282-291.

Kambarova D.K., Belyayev V.V., Volkova A.V. et al. (2010) Methods of nonlinear dynamics in estimation of electroencephalograms of healthy people and of patients with epilepsy. *J. of Evolutionary Biochemistry and Physiology*, **46 (5):** 501-510.

Kempermann G., Gast D., Gage F.H. (2002) Neuroplasticity in old age: Sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann. Neurol*., **52 (2):** 135 p.

Klimesch W., Doppelmayr M., Hanslmayr S. (2006) Uppler alpha ERD and absolute power: their meaning for memory performance. *Prog. Brain Res*., **159:** 151p.

Kozlov V.I. (1982) Study of the longevity problems. In: *Longevity phenomenom.* Moscov: Nauka, p. 5-12

Kulaichev A.P. (2007) Computer electrophy-siology and functional diagnostics. M.: Forum-Infra, 640 p.

Kuznetsova S.M. (2008) On the question of the mechanisms of longevity: what is the secret of longevity? What determines the longevity phenomenon? Zdorove dovqolittya. *Mediz Antiaging. Healthy aging*, **2 (02):** 40-43

Liddell B.J., Paul R.H., Arns M. et al. (2007) Rates of decline distinguish Alzheimer's disease and mild cognitive impairment relative to normal aging: integrating cognition and brain function. *J. Integr. Neurosci*., **6(1):** 14 p

Marx E., Deutschlander A., Stephan T. et. al (2004) Eyes open and eyes closed as rest conditions, impact on brain activation patterns. *Neuroimage*, **4:** 1818 p.

McEvoy L.K., Pellouchoud E., Smith M.E., Gevins A. (2001)Neurophysiological signals of working memory in normal aging. *Brain Res.: Cogn.Brain Res*., **11:** 363 p.

Moretti D. V., Frisoni G. B., Binetti G., Zanetti O. (2012) Mild cognitive impairment and quantitative EEG markers: Degenerative versus vascular brain damage. *Advances in Clinical Neurophysiology*, p. 171-194.

Polunina A.G. (2012) Encephalogram charac-teristics in the assessment of cognitive functions. *Nevrol. Psychiatric n.a. S.S. Korsakova*, **112(7):** 62-70.

Razoumnikova O.M. (2003) Reflection of the intelligence structure in the spatiotemporal features of the baseline EEG. *Human Physiology*. **29 (5):** 115-122.

Van Cott A.C. (2002) Epilepsy and EEG in the elderly. *Epilepsia*, **43(3):** 94-102.

Volf N.V., Tarasova I.V. (2010) The relationships between EEG Θ and β oscillations and the level of creativity. *Human Physiology*, **36 (2):** 132-138.

Volf N.V., Glukhih A.A. (2011) Background electrical activity of brain in healthy mental aging. *Human Physiology*, **37(5):** 51-60.

Vysata O., Kukal J., Prochazka A., Pazdera L., Valis M. (2012) Age-related changes in the energy and spectral composition of EEG. *Neuropsychology*, **14(1):** 63-67.

Zenkov L.R. (2010) Clinical epileptology. M.: Med. inform agency, p. 123-129.

Azərbaycanın müxtəlif rayonlarında yaşayan uzunömürlülərdə baş beynin bioelektrik fəallığının müqayisəli xarakteristikası

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Məqalədə müasir kompüter elektroensefaloqrafiyası metodundan istifadə etməklə Azərbaycanın cənub zonasının uzunömürlülük indeksi yüksək olan Lənkəran (ərazisi əsasən düzənlik) və Lerik (dağlıq) rayonlarında yaşayan uzunömürlülərdə fon elektroensefaloqrammanın spektral-tezlik analizinin müqayisəli nəticələri verilmişdir. Aparılmış elektofizioloji tədqiqat işi Azərbaycanın müxtəlif coğrafi ərazilərindəki uzunömürlü populyasiyalarında uzunömürlülərin elektroensefaloqrammasında (EEQ) oxşar və fərqli xüsusiyyətlərin olduğunu göstərir. Uzunömürlülərin fon EEQ-nin müqayisəli analizi əsasında hər iki rayon uzunömürlülərində baş beynin funksional vəziyyətinin qabıqaltı strukturların qabığın fəallığına tormozlayıcı (delta- və teta-ritmlərin göstəricilərinin dominantlığı zəminində) təsiri ilə müşayiət olunan aşağı fəallıqla xarakterizə edildiyi, ancaq Lerik rayonunda yaşayan uzunömürlülərdə Lənkəran uzunömürlülərindən fərqli olaraq mezensefal retikulyar formasiyanın baş-beyin qabığına aktivləşdirici təsirinin kompensator yüksəlməsi müəyyənləşdirilmişdir. Uzunömürlülərin EEQ-də ümumi cəhətlərlə yanaşı fərqliliyin olması beynin kompensator mexanizmlərinin fəallaşmasının müxtəlif istiqamətini göstərir ki, bu da uzunömürlülərdə fizioloji yaş dəyişikliyinə məruz qalan mərkəzi sinir sisteminin neyron şəbəkəsinin yenidən təşkili və beynin aktiv fəaliyyətini təmin etmək üçün daha çox resursun işə cəlb edilməsi haqqındakı fərziyyəni irəli sürməyə imkan verir.

Açar sözlər: *Uzunömürlülər, elektroensefaloqramma, spektral analiz, spektral güc, indeks, baş beynin funksional vəziyyəti*

Сравнительная характеристика биоэлектрической активности головного мозга у долгожителей, проживающих в различных районах Азербайджана

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В статье представлены сравнительные результаты спектрально-частотного анализа фоновых электроэнцефалограмм, полученных методом современной компьютерной электроэнцефалографии у долгожителей Ленкоранского (в основном равнинного) и Лерикского (горного) районов южной зоны Азербайджана, характеризующихся высоким индексом долголетия. Электрофизиологическое исследование показало схожие и отличительные особенности в ЭЭГ коры мозга у долгожителей в популяциях различных географических районов Азербайджана. Сравнительный анализ фонового ЭЭГ у долгожителей обоих районов выявил низкую активность функционального состояния головного мозга, сопровождающуюся тормозящим влиянием подкорковых структур на активность коры. Тем не менее, показано, что по сравнению с долгожителями Ленкоранского района, в ЭЭГ мозга долгожителей Лерикского района преобладает активирующее влияние мезенцефальной ретикулярной формации на кору головного мозга, что можно объяснить различием уровня активности компенсаторных механизмов мозга у изучаемых долгожителей обоих районов. Предполагается, что полученные физиологические изменения в ЭЭГ мозга могут быть связаны с реорганизацией нейронной сети с привлечением больших ресурсов для обеспечения активной деятельности мозга.

Ключевые слова: *Долгожители, электроэнцефалограмма, спектральный анализ, спектральная мощность, индекс, функциональное состояние мозга*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 17-22 (2020)

Impact of different types of crude oil on embryonic neurotransmitters

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Accepted for publication: 22 May 2020

The effect of crude oil on contents of different neurotransmitters during sturgeon embryogenesis has been studied. Experiments have shown that different type of crude oils produce diverse responses of neurotransmitter systems, supporting their role as a target for developmental toxicity of crude oil.

Keywords: *Embryonic development, crude oil, neurotransmitters*

INTRODUCTION

In spite of commitment of modern hi-tech society to renewable sources of energy, the crude oil is still the major energy source and is expected to remain so for very long forthcoming time. The growth of world energy consumption is the principal driver behind massive offshore upstream investments and expansion of oil and gas transportation networks (BP Statistical Review of World Energy, 1975-2015). Alongside the coastal-based oil industry facilities (downstream sector) and natural seepage, the offshore activities and midstream oil operations contribute to massive discharge of crude oil into marine environment that is the global hot-button issue (Anderson et al., 2012). Ecosystem's response to crude oil pollution depends mainly on two factors: the type of oil and the ecological characteristics of the affected area. Complex chemical composition of crude oil determines biological and physical properties, the fate and behavior of oil in marine waters. Environmental abiotic characteristics (temperature, water chemistry and salinity, oxygen level, agricultural and urban pollutions) interfere heavily with oil toxicity making the impact on ecological networks unpredictable. The other important aspect is marine organisms' sensitivity to crude oil pollution, which varies between species, ages (developmental stage) and individual properties.

The phenotypic expressions of crude oils toxicity are multiple and include carcinogenicity, immune deficiency and high disease susceptibility, behavioral impairment, endocrine disruptions, reproduction effect, developmental malformation etc. Target specificity effect of different types of oil is a subject for debate because the underlying mechanisms of these effects still need to be elucidated (Dubansky et al., 2013; The Royal Society of Canada expert panel, 2015).

Early developmental stages are particularly vulnerable to crude oil exposure (Carls et al., 2009; Hicken et al., 2011; Hodson, 2017; Incardona, 2017; Meador et al., 2019). Embryogenesis is highly orchestrated multistage process with many players to regulate developmental progression including neurotransmitters. Monoamine neurotransmitters, besides their regulation of cognitive and behavioral functions on adult life, are involved in very complex processes of embryo pattern formation via controlling gene expression program (Sullivan et al., 2016). Interference of certain individual crude oil components with neurotransmitters' functions can trigger time- and space- improper signaling cascades resulted in acute (lethality) or delayed (disease) pathological outcomes.

Based on the said premises, the work was undertaken to study the effect of different types of crude oil on expression pattern of neurotransmitters during embryogenesis.

MATERIAL AND METHODS

The experiments were conducted on the embryos of *Acipenser stellatus*, obtained from the

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broodstock at Hylly Sturgeon hatchery. The fertilization of fish eggs was performed using the semidry techniques in small volume of solution, contained either Azeri Light oil from Chirag oilfield (light crude oils, 32º API gravity), or Neft Dashlari oil (medium crude oils, 28º API gravity) at a concentration 100 ppm. On completion of fertilization procedure (about 2-3 min) and just before the eggs become sticky, they were evenly distributed in Petri dishes and incubated in a corresponding medium until hatching under natural light/dark cycle at a room temperature (24ºC). Controls were fertilized and incubated in clean water.

The identification of embryonic stages and assessment of the rate, asynchrony and defects of developmental progression were performed under stereotaxic microscope, based on chronology of advent of specific features, described by Detlaf (Детлаф и др., 1981).

At 4 developmental stages: before fertilization (bf), blastula (bl), gastrula and neurula the randomly selected embryos were sampled to measure serotonin (5-HT), dopamine (D) and noradrenaline (NA) content by indirect ELISA using polyclonal antibodies to those monoamines. The data for norepinephrine were expressed in terms of optical density. The concentrations of serotonin and dopamine were determined by interpolation of the data from standard curves, basing on the obtained values of optic densities. t-Student's test was applied to evaluate differences between the averaged values of the groups.

RESULTS

During early developmental progression each of three neurotransmitters (NTs) in control eggs has shown the same trends in the stage-dependent expression (Fig. 1). The highest level of NTs was observed in mature oocytes before fertilization (that data was treated as the starting point for dynamic assessment). Shortly after fertilization it starts dropping and progressively declines throughout blastula and gastrula until neural stage, when the level starts going up.

Noteworthy, the amplitudes of fluctuations in concentrations between stages were quite different for serotonin vs. catecholamines. In particular, it was greater for NA and D and mild for 5-HT.

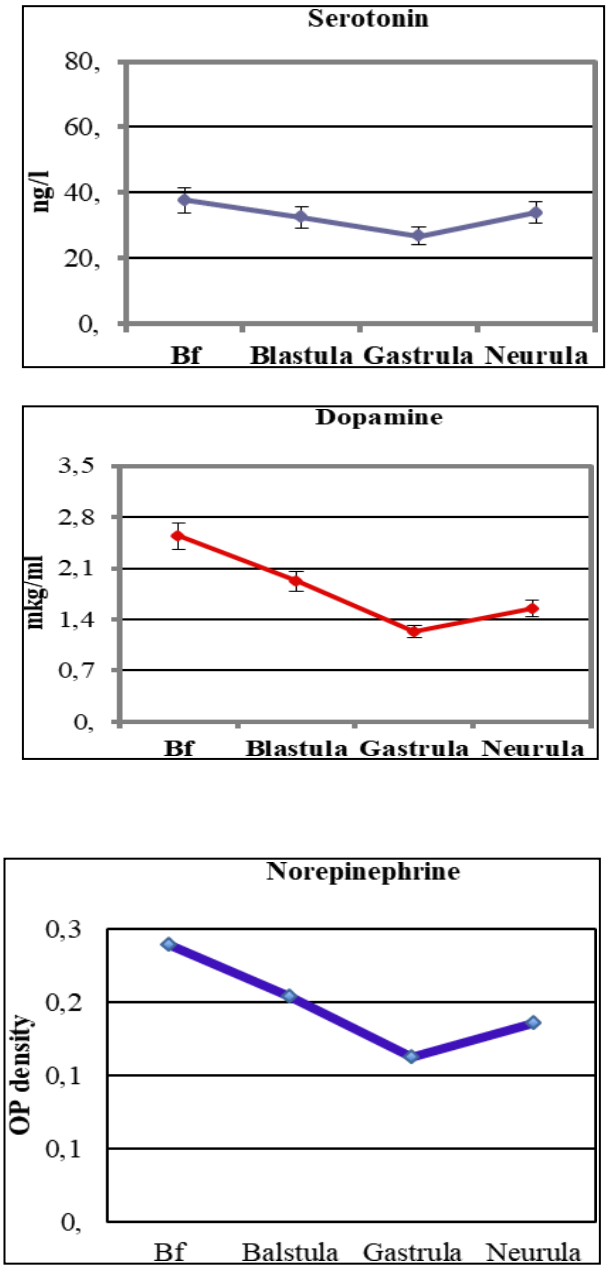

Figure 1. Changes in neurotransmitters level through early stages of embryonic development.

Exposure of fish eggs to both types of crude oil significantly downregulated of dopamine and norepinephrine and slightly depressed 5- HT in mature oocytes (Fig. 2-4). At subsequent stages of development the different types of oil produced different responses of neurotransmitter systems.

During incubation in the medium, containing the oil from the deposit Neft Dashlari, changes of

the levels of all three NTs in the fish eggs decreased markedly, though the stage-dependent fluctuations were similar to the controls up to the neurula stage, when the expected upregulation of NTs did not occur.

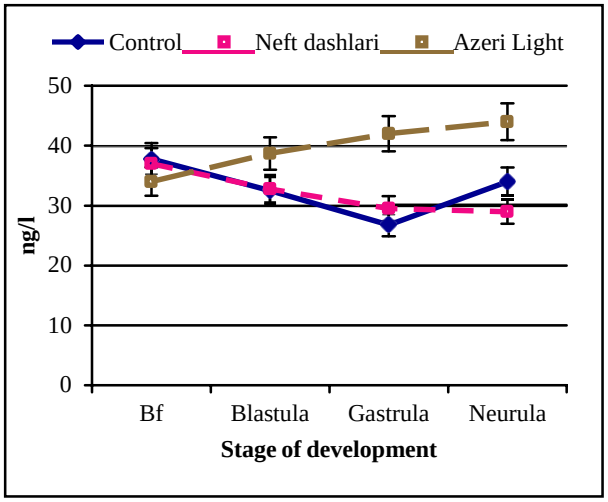

Figure 2. Changes in Serotonin level through early stages of embryonic development under different types of crude oil exposure.

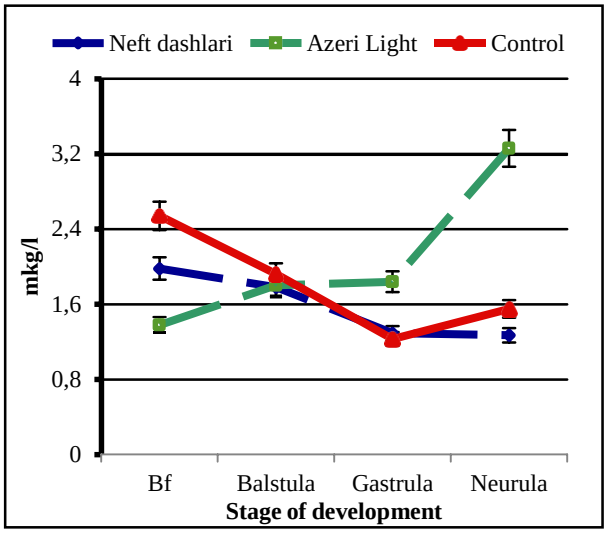

Figure 3. Changes in Dopamine level through early stages of embryonic development under different types of crude oil exposure.

Exposure to Azeri light oil caused upregulation of neurotransmitters at all stages of embryonic development. The effect was prominent on both NA via increasing content and flattering its fluctuation, and DA via bringing about twofold increase at the neurula stage in comparison with the controls ($p < 0.05$; Fig. 3).

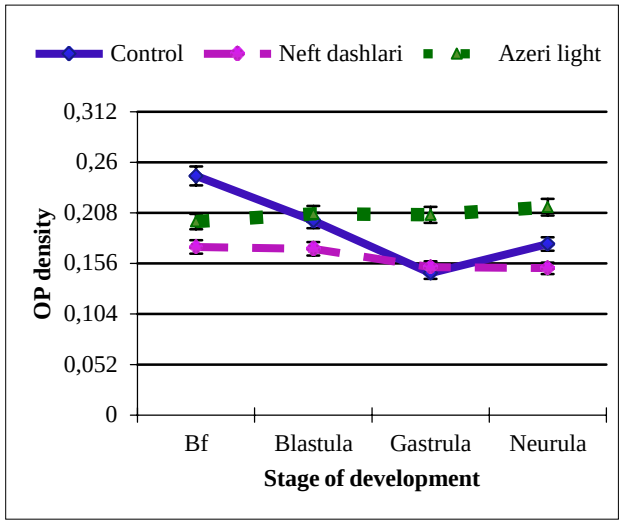

Figure 4. Changes in Norepinephrine level through early stages of embryonic development under different types of crude oil exposure

On the whole, exposure to different types of oil resulted in changes of neurotransmitter's expression pattern: Azeri light oil had stimulating effect, whereas the Neft Dashlari oil decreased neurotransmitters content. Besides, the higher hatching rate and best survival were documented for Azeri light oil. The frequently encountered abnormalities in newly hatched larva (head and spinal malformation, deviation from bilateral symmetry, yolk sac deformation, body shortening etc.) were very common for embryo toxic effects of both crude oils.

DISCUSSION

Crude oil is the naturally occurring fossil fuel and very common pollutant in marine environment due to its natural seepage and anthropogenic spills (Anderson et al., 2012). In spite of extremely complex chemical compositions, the crude oils mainly contain the same 4 major classes of compounds: Saturates, Aromatics, Resins and Asphaltenes (acronym SARA) and very small concentration of non-hydrocarbon components like heavy metals, sulphur etc. (The Royal Society of Canada expert panel, 2015). The different pro-

portions between classes and the individual constituents determine all spectrum of the physical and chemical properties, the environmental behavior and toxicity risks of crude oils.

According to scale for gravity of American Petroleum Institute (API), there are 3 types of crude oil: light (°API>31.1; medium (31.1>°API>22.3) and heavy (°API<22.3). API roughly indicates the susceptibility of crude oil to different weathering processes, affecting its behavior, fate and chemical compositions of residues after leak into the aquatic environment.

The light type of oil contains predominantly saturated hydrocarbons and is rich in low weight aromatic; other families of hydrocarbons are present in rather low concentrations. From medium to heavy oils the abundance of resins and asphaltenes prominently increased. Commonly, the saturates are considered as the least toxic and easily biodegradable compounds; the low weight aromatics (especially, benzene, toluene, ethylbenzene, xylene - BTEX acronym) are treated to be the most toxic, water soluble components which are responsible for acute toxicity/lethality. The polyaromatic hydrocarbons (PAH), resins and asphaltenes are progressively less soluble, more polar, and typically are resistant to biodegradation and have tendency to precipitate out. Their environmental persistence contributes to sub-lethal/chronic toxicity (The Royal Society of Canada expert panel, 2015).

There are no specific signs and/or target organs, which are attributed to crude oil effects at adult life stage, however multiple manifestations of toxicity cannot be treated simply as a sum (combination) of individual compound-specific effects.

It is postulated, that the lipophilic components of crude oils and/or products of their metabolism via interaction with receptors or other responsive elements, affect the intracellular signaling pathways resulting in numerous pathological (pathophysiological) outcomes, especially if these changes occur during the early stages of embryonic development (Carls et al., 2009; Hicken et al., 2011; Hodson, 2017; Incardona, 2017; Meador et al., 2019; The Royal Society of Canada expert panel, 2015).

In our experiments changes on embryo neurotransmitters' patterns under crude oil exposure have been detected.

The persistence of neurotransmitters in mature oocytes and at all stages of embryogenesis suggested their participation in key developmental processes: cell movement, proliferation, fate determination and tissue differentiation (Sullivan et al., 2016; Thomas et al., 1995; Yavarone et al., 1993).

First, they may be responsible for cell-cell or cell-matrix communications, serving as morphogen or gradient shaping signal for other morphogens. Crude oil-induced changes in neurotransmitters expression reflect changes in their concentration gradients, which is crucial for proper spatio-temporal sequencing of developmental events. This supposed to be underlying mechanism of embryo lethality, malformation and adult life pathology, depending on the stage to be affected.

Noteworthy, that the most typical expression of developmental toxicity of crude oil is the craniofacial, body axis and cardia malformations. The same effects were described under pharmacological inhibition of serotonin and dopamine activities during certain stages of embryogenesis. There is a great deal of research that confirms the participation of serotonin and dopamine in morphogenesis of craniofacial structures (Greene et al., 2018). Other studies indicated the essential role for serotonin and norepinephrine in myocardial (pacemaker and conduction system) development (Thomas et al., 1995; Yavarone et al., 1993).

The more recent researches have shown that serotonin play a pivotal role in left-right pattering (lateralization of tissue and organs): its accumulation on right side of embryo epigenetically represses (through the histone deacetylase binding partner Mad3) transformative factor *nodal* on that side with triggering the laterality pathway on the left (Fukumoto et al., 2005). These data suggest that changes in gradients of serotonin and catecholamine neurotransmitters underlie the malformation development under to exposure crude oil.

Neurotransmitters are fundamentally involved in all steps of development of nervous system; so many pathological conditions (cognitive and behavioral disturbances) may arise from the impairment of neurotransmitter-mediated neurogenesis under early developmental exposure to the crude oil.

Besides the obvious appearance of crude oils-induced toxicity, there is another important aspect to mention. The intrusions of crude oil components or its metabolites into extra- and intra-cellular microenvironment, interfere with the embryo's developmental program, may also alter epigenetic landscape, especially during erasure of DNA methylation and re-programming normally occurring in mid-blastula stage (Mhanni et al., 2004; Perera et al., 2011; Reamon-Buettner et al., 2007; Szyf, 2011). Aberrant epigenome, responsible for late onset diseases (e.g. reproductive, immune, behavioral function), can be inherited across generations and potentially affects reproduction and survival success, resulting in decrease population density.

The last, but not the least: there are not many receptor- or pathway-based data to explain the crude oil toxicity. That is why many authors suggested that commonly observed toxic effect of crude oils should be considered as the non-specific physiological response. In our experiments different types of crude oil produced diverse response of neurotransmitters systems during embryogenesis, supporting the idea of "chemical structure-physiological response" that is the subject for further research to be studied.

REFERENCES

Детлаф Т.А., Гинзбург А.С., Шмальгаузен О.И. (1981) Развитие осетровых рыб. М: Наука.

Anderson, C.M. Mayes, M., and LaBelle, R.P. (2012) Update of occurrence rates for offshore oil spills. Bureau of Ocean Energy Management OCS Report 2012-069. Herndon, VA: Bureau of Ocean Energy Management.

BP Statistical Review of World Energy (1975-2015, annual reports). London: Whitehouse Associates. Retrieved from http://www.bp.com/statisticalreview

Carls M.G., Meador J.P. (2009) A perspective on the toxicity of pertogenic PAHs to developing fish embryos related to environmental chemistry. *Human and Ecological Risk Assessment,* **15(6):** 1084-1098.

Dubansky B., Whitehead A., Miller J.T., Rice C.D., Galvez F. (2013) Multitissue molecular, genomic, and developmental effects of the Deepwater Horizon oil spill on resident Gulf killifish (Fundulus grandis). *Environ SciTechnol*., **47(10):** 5074–5082.

Fukumoto T., Kema I.P., Levin M. (2005) Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. *Curr. Biol.,* **15:** 794-803; PMID:15886096; http://dx.doi.org/10.1016/j.cub.2005 .03.044

Greene R., Pisano M.M. (2018) Serotonin signaling as a target for craniofacial embryotoxicity. In: *Handbook of Developmental Neurotoxicology (Second Edition)*, W.Slikker, M.G.Paule, C.Wang (eds). Academic Press, p. 65-74, ISBN 9780128094051, https://doi.org/10.1016/B978-0-12-809405-1.00006-7.

Hicken C.E., Linbo T.L., Baldwin D.H., Willis M.L., Myers M.S., Holland L., Larsen M., Stekoll M.S., Rice S.D., Collier T.K., Scholz N.L., Incardona J.P. (2011) Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proc. Natl. Acad. Sci. USA,* **108(17):** 7086-7090.

Hodson P.V. (2017) The toxicity to fish embryos of PAH in crude and refined oils. *Arch Environ Contam. Toxicol.,* **73:** 12-18; https://doi.org/10.1007/s00244-016-0357-6

Incardona J.P. (2017) Molecular mechanisms of crude oil developmental toxicity in fish. *Arch Environ. Contam. Toxicol.* **73:** 19-32; https://doi.org/10.1007/s00244-017-0381-1

Meador J.P., Nahrgang J. (2019) Characterizing crude oil toxicity to early-life stage fish based on a complex mixture: are we making unsupported assumptions? *Environmental Science & Technology,* **53 (19):** 11080-11092

Mhanni A.A., McGowan R.A. (2004) Global changes in genomic methylation levels during early development of the zebrafish embryo. *Dev. Genes Evol.,* **214(8):** 412-417.

Perera F., Herbstman J. (2011) Prenatal environmental exposures, epigenetics, and disease. *Reprod. Toxicol.,* **31(3):** 363-373.

Reamon-Buettner S.M, Borlak J. (2007) A new paradigm in toxicology and teratology: altering gene activity in the absence of DNA sequence variation. *Reprod. Toxicol*., **24(1):** 20-30.

Sullivan K.G., Levin M. (2016) Neurotransmitter signaling pathways required for normal develop-

ment in *Xenopus laevis* embryos: a pharmacological survey screen. *J. Anat*., **229(4):** 483-502. doi:10.1111/joa.12467

Szyf M. (2011) The implications of DNA methylation for toxicology: toward toxicomethylomics, the toxicology of DNA methylation. *Toxicol Sci*., **120(2):** 235-255

The Royal Society of Canada expert panel. (2015) The behaviour and environmental impacts of crude oil released into aqueous environments. https://www.cepa.com/wp-content/ uploads/2014/01/OIW-Report.compressed.pdf

Thomas S., Matsumoto A., Palmiter R. (1995) Noradrenaline is essential for mouse fetal development. *Nature*, **374:** 643–646 https://doi.org/10.1038/374643a0

Yavarone M.S., Shuey D.L., Tamir H. et al. (1993), Serotonin and cardiac morphogenesis in the mouse embryo. *Teratology*, **47:** 573-584. doi:10.1002/tera.1420470609

Xam neftin müxtəlif növlərinin embriogenezdə monoaminlərin dinamikasina təsiri

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Tədqiqatlarda uzunburunun (*Acipenser stellatus*) embrional inkişaf dövründə neyrotrasmitterlərin dinamikası öyrənilmişdir. Aşkar olunub ki, xam neftin növündən asılı olaraq rüşeymdə monoaminlərin qradienti dəyişir və bu dəyişiklər neftin toksiki təsirinin əsasında dura bilər.

Açar sözlər: *Embrional inkişaf, xam neft, neyrotransmitterlər*

Влияние различных типов нефти на уровень нейротрансмиттеров в эмбриогенезе

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Изучено влияние различных типов нефти на динамику экспрессии нейротрансмиттеров в эмбриогенезе севрюги *Acipenser stellatus*. Предполагается, что изменение их концентрационного градиента может лежать в основе токсического действия нефти

Ключевые слова: *Эмбриональное развитие, сырая нефть, нейротрансмиттеры*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 23-29 (2020)

Transgenerational transmission of prenatal hypoxia-induced changes of two enzymes in the brain structures of rat progeny

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Accepted for publication: 12 March 2020

The article concerns the problem of transgenerational transmission of the changed activities of the enzymes of pyrophosphatase and succinate dehydrogenase in the rats. The experiments were carried out on 6-month-old female Wistar rats and their progeny at the age of 17 and 30 days. The gestated rats, whose fetus were at the stage of organogenesis, were subjected to 20-minute daily hypoxia (gas mixture of 90% nitrogen and 10% oxygen) for 5 days. The rat pups born from these females, were decapitated and fragments of the orbital, sensorimotor and limbic cortex, hypothalamus and cerebellum were taken from their brains and mitochondrial and cytosol fractions were fractionated. In the mitochondrial fractions of the all studied structures, significant downregulation of the pyrophosphatase activity was found in the 17-day-old rat pups, though in the cytosol fraction it was noted only in the orbital cortex. In the mitochondrial (except for the orbital and limbic cortex) and cytosol fractions of the all studied structures of the 30-day-old rats, downregulation of pyrophosphatase as well was observed. On the contrary, in the 17-day-old rat pups in the mitochondrial fractions of the all studied structures, prominent upregulation of the activity of succinate dehydrogenase with its simultaneous downregulation in the cytosol fractions of the orbital and limbic cortex, and cerebellum was revealed. In the 30-day-old rat pups, significant upregulation of this enzyme activity was observed in the cytosol fractions of the all studied structures, whereas in the mitochondrial fractions (except for sensorimotor cortex) no changes were noted. It is concluded that a transgenerational transmission of the altered activity of two enzymes occur, apparently due to epigenetic changes in the activity of the corresponding genes.

Keywords: *Pyrophosphatase, succinate dehydrogenase, rats, brain structures, transgenerational transmission.*

INTRODUCTION

Presently the problem of a risk of transgenerational transmission of pathological states attracts significant attention of scientists. It has been shown that several types of pathological states can be transmitted from one generation of the animals to the next one (Bohacek and Mansuy, 2013; Steenwyk et al., 2018). This problem has both scientific and significant medical aspects and should be the subject of intensive multidisciplinary studies. The earlier conducted studies revealed that offspring of the female Wistar rats, exposed to hypoxia during the organogenesis period of gestation, in achieving the postnatal ages of 17 and 30 days, had significant upregulation of the activities of some enzymes, in particular pyrophosphatase and succinate dehydrogenase (Abiyeva, 2015; Rashidova et al., 2019).

The goal of the present studies concludes in analysis of possibilities of transmission of the changes of the activities of enzymes of pyrophosphatase and succinate dehydrogenase of the brain structures of the Wistar rats, subjected to hypoxia during the organogenesis period of gestation, to next generations.

MATERIALS AND METHODS

The studies were carried out on the Wistar 6-month-old female pregnant rats and their progeny at the ages of 17 and 30 days. The gestated female

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rats, whose fetus was at the stage of fetal organogenesis (6-16 days of gestation), were subjected to 20-minute hypoxia (gas mixture of 90% nitrogen and 10% oxygen) daily, for 5 consequent days. Their progeny, 17-day-old (n=4) and 3-month-old (n=4) rat pups were sacrificed and orbital, limbic and sensorimotor cortex, hypothalamus and cerebellum were removed, homogenized in 0.25 M sucrose solution at a ratio of 1:9 and centrifuged at a 20,000 g for 20 min to separate mitochondrial (precipitate) and cytosol (supernatant) fractions.

Definition of activity of succinate dehydrogenase. Incubation media was prepared by mixing 1 ml of 0.1 M phosphate buffer, 1 ml of 0.1 M succinic acid, 1 ml of 25 mM EDTA and 1 ml of 150 mM of sodium azide and bringing pH value of the mixture to pH7.8. The experimental tubes were poured with 140 µL of the incubation media, while the control tubes were poured with 2 ml of 20% solution of three-chlorine-acetic acid (TCAA); thereafter 0.5 mL of mitochondria suspension were added to all tubes and incubate for 5 min at room temperature. The reaction was launched by addition of 0.1 mL of 25 mM potassium ferrocianide and the samples were incubated at 30ºC for 10-15 min. The reaction was stopped by cooling the samples and adding 2 ml of 20% solution of TCAA into the experimental tubes. All samples were centrifuged, supernatants were saved and their extinction was measured on the spectrophotometer at a wavelength of 420 nm.

Definition of activity of pyrophosphatase. Incubation media was prepared by mixing 1 mL of 1mM PPi, 1 mL of 4 mM $MgCl_2$, 1 mL of 0.1 mM EDTA, and 1 mL of 0.05 M tris-HCl buffer and bringing pH value of the mixture to pH 7.4. The experimental and control tubes were poured with 1 mL of incubation media and 20 µL of a sample and only in the control tubes 166 µL of 20% solution of TCAA were added. All samples were incubated under 25ºC for 30 min and then 166 µL of 20% solution of TCAA was added to the experimental tubes too. The samples were incubated under ambient temperature for 10 min. The obtained extracts in an amount of 20 µL were added to the tubes containing 1 mL of the second incubation mixture containing 30 mL of o.1 M acetate buffer, 3 mM of 1% molibdenic acidic ammonium and 3 mL of 1% ascorbic acid, incubated under ambient temperature for 10 min and the extinction was measured on a spectrophotometer at wavelength of 660 nm.

The differences between groups were evaluated with application of Student's t-criterion.

RESULTS

The results of measuring the activities of succinate dehydrogenase and pyrophosphatase in the progeny of the female rats, subjected to 10% hypoxia during gestation period, at the stage of organogenesis, revealed significant changes in their specific activities in different brain structures. In the 17-day-old rat pups significant downregulations of the specific activities of pyrophosphotase in the mitochondrial fractions of the orbital (experimental: 16.6±1.4 vs. control: 53.7±1.1, $p<0.001$), sensorimotor (14.6±1.7 vs. 28.4±1.2, $p<0.01$), limbic cortex (13.1±1.5 vs. 25.6±1.7, $p<0.01$), hypothalamus (5.3±0.6 vs. 27.7±1.4, $p<0.001$) and cerebellum (6.0±0.9 vs. 20.4±1.8, $p<0.01$) were noted (Fig. 1).

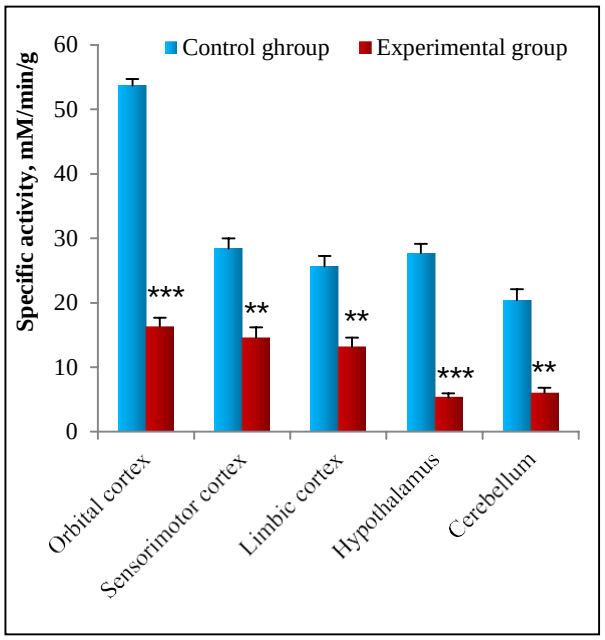

Figure 1. Changes of the pyrophosphatase specific activities in the mitochondrial fractions of the brain structures of the 17-day-old rat pups.
** $p<0.01$, *** - $p<0.001$.

In the rat pups of the same age in the cytosol fractions the pronounced downregulation of the

specific activities of pyrophosphotase was revealed only in the orbital cortex (17.2±1.5 vs. 28.4±1.0, p<0.01) and hypothalamus (6.3±0.7 vs. 15.8±0.9, p<0.01), though in the other studied brain structures the differences in the enzyme activities were non-significant.

In the 30-day-old rat pups, born from the female rats, subjected during gestation period to 10% hypoxia, noticeable down-regulation of the specific activities of pyrophosphotase in the mitochondrial fractions of the sensorimotor cortex (5.3±0.7 vs. 39.9±1.1, p<0.001), limbic cortex (23.2±2.1 vs. 38.8±1.2, p<0.01), hypothalamus (1.9±0.2 vs. 29.3±0.9, p<0.001) and cerebellum (6.3±0.6 vs. 31.2±0.8, p<0.001) was found, while in the orbital cortex the differences were non-significant (Fig. 2).

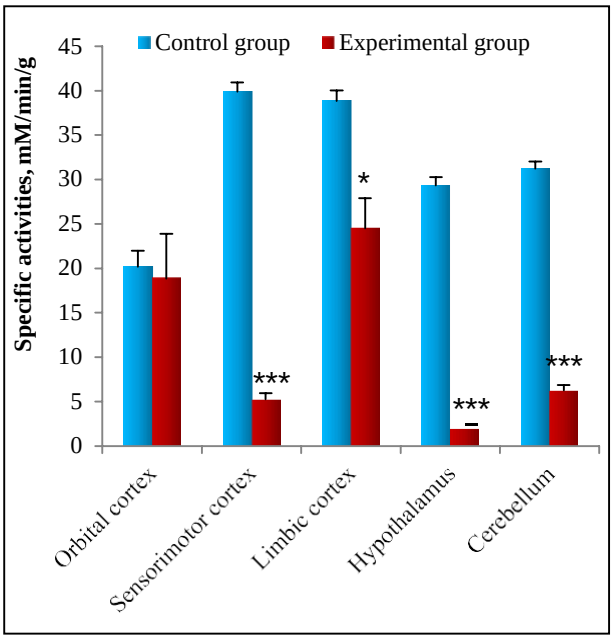

Figure 2. Changes of the pyrophosphatase specific activities in the mitochondrial fractions of the brain structures of the 30-day-old rat pups.
* p<0.05, *** - p<0.001.

In the rat pups of this age group in the cytosol fractions the pronounced downergulation of the specific activities of pyrophosphotase was observed in the orbital (17.1±2.1 vs. 65.6±1.3, p<0.001), limbic cortex (4.3±0.3 vs. 9.0±0.8, p<0.01), hypothalamus (10.7±0.4 vs. 31.8±1.3, p<0.001) and cerebellum (10.7±1.2 vs. 58.7±1.5, p<0.001; Fig. 3).

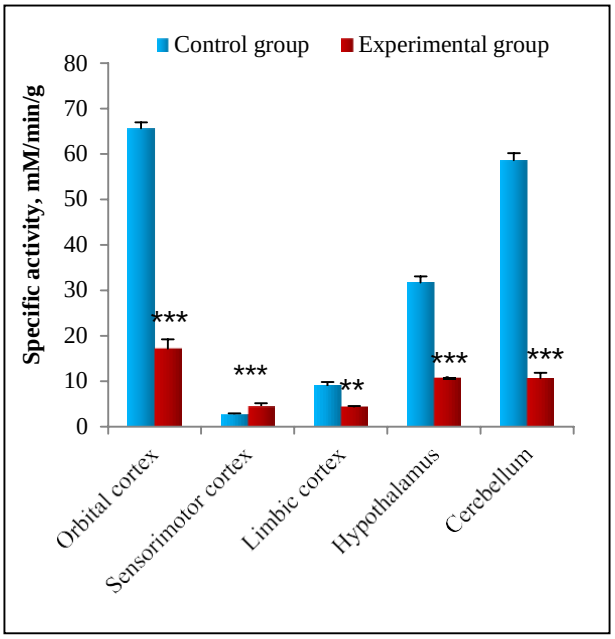

Figure 3. Changes of the pyrophosphatase specific activities in the cytosol fractions of the brain structures of the 30-day-old rat pups.
** p<0.01, *** - p<0.001.

Analysis of the specific activities of succinate dehydrogenase of the brain structures of the progeny of the female rats, subjected to 10% hypoxia during gestation period, at the stage of organogenesis, in comparison to pyrophosphatase activities, revealed quite different character of changes. In the 17-day-old rat pups significant up-regulation of the specific activities of this enzyme in the mitochondrial fractions of the all studied brain structures was noted. In particular, in the orbital cortex of the rat pups its values were 38.7±4.3 vs. 17.1±1.1 (p<0.01), in the sensorimotor cortex – 48.5±2.6 vs. 30.2±1.0 (p<0.01), in the limbic cortex – 34.2±2.2 vs. 23.5±1.3 (p<0.05), in the hypothalamus – 23.7±0.7 vs. 2.7±0.1 (p<0.001) and cerebellum – 28.7±4.2 vs. 4.2±0.36 (p<0.01; Fig. 4).

At the same time, in the cytosol fractions, in opposite to the mitochondrial fractions, some downregulation of the enzyme activities was revealed; in the orbital cortex – 28.0±2.3 vs. 42.3±1.8 (p<0.01), in the limbic cortex – 20.7±2.3 vs. 29.5±0.5 (p<0.05) and in the cerebellum – 12.2±1.5 vs. 17.9±1.2 (p<0.05; Fig. 5).

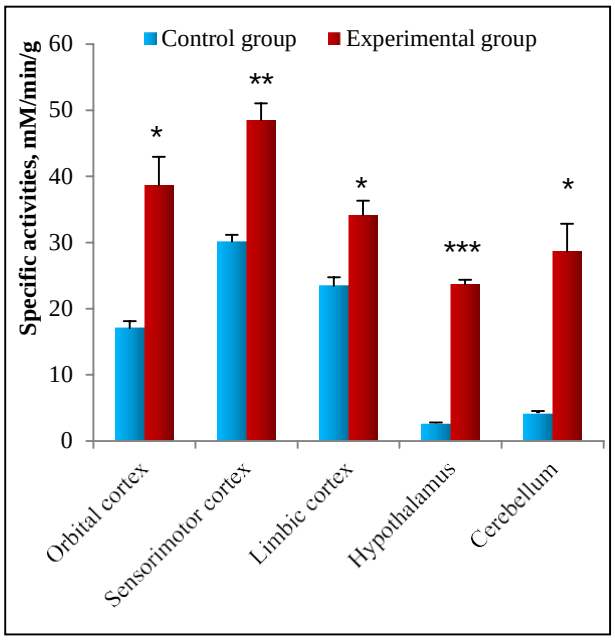

Figure 4. Changes of the specific activities of succinate dehydrogenase in the mitochondrial fractions of the brain structures of the 17-day-old rat pups. *- p<0.05, ** - p<0.01, *** - p<0.001.

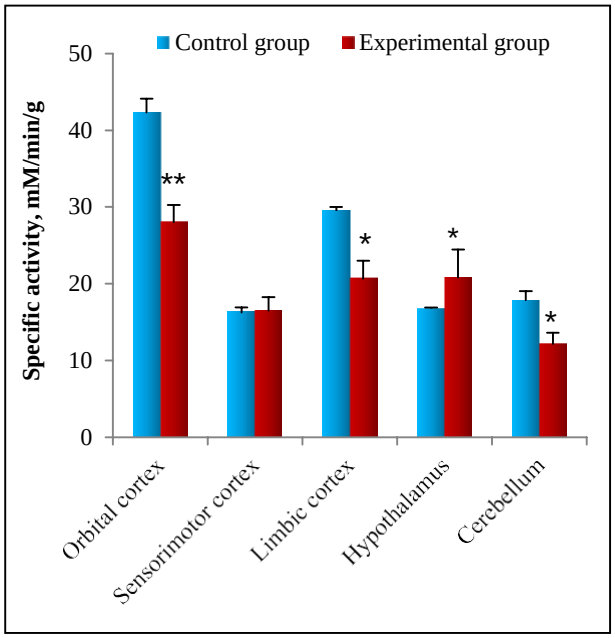

Figure 5. Changes of the specific activities of succinate dehydrogenase in the cytosol fractions of the brain structures of the 17-day-old rat pups. *- p<0.05, ** - p<0.01.

In the 30-day-old rat pups, born from the female rats, subjected to 10% hypoxia during gestation period, only in the mitochondrial fraction of the sensorimotor cortex some upregulation of succinate dehydrogenase (24.3±1.2 vs. 14.4±1.3, p<0.01) was observed, whereas in other studied brain structures (orbital and limbic cortex, hypothalamus and cerebellum) no significant changes of the enzyme activity were found. Conversely, in the cytosol fractions of the all studied brain structures of the rat pups of this age group significant upregulation of the enzyme's activities was revealed: in the orbital cortex – 33.5±3.3 vs. 7.9±1.1 (p<0.01), in the sensorimotor cortex – 50.5±4.3 vs. 31.3±2.1 (p<0.05), in the limbic cortex – 23.7±3.2 vs. 7.3±1.1 (p<0.01), in the hypothalamus – 48.2±4.3 vs. 6.6±1.0 (p<0.001) and in the cerebellum - 29±2.7 vs. 9.3±1.0 (p<0.01; Fig. 6).

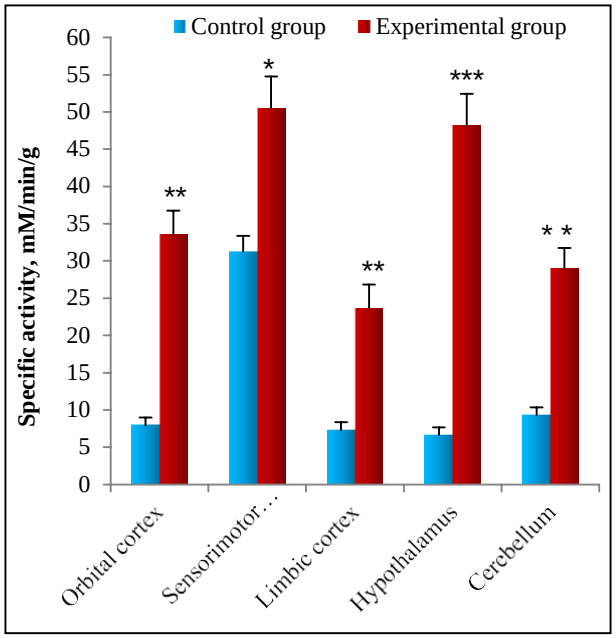

Figure 6. Changes of the specific activities of succinate dehydrogenase in the cytosol fractions of the brain structures of the 30-day-old rat pups. *- p<0.05, ** - p<0.01, *** - p<0.001.

Hence, the results of the studies indicate to induction of significant opposite directional changes of the activities of pyrophosphatase and succinate dehydrogenase in the brain structures of the first progeny of the female rats, undergone 10% hypoxia at the organogenesis stage of their gestation period. In this case, in the 17-day-old rat pups downregulation of pyrophosphatase activity was observed in the mitochondrial fractions of the or-

bital, sensorimotor and limbic cortex, hypothalamus and cerebellum, while in the cytosol fraction such downregulation was noted only in the orbital cortex. Along with it, in the 30-day-old rat pups downregulation of pyrophosphatase activities were observed both in the mitochondrial (in exception for the orbital and limbic cortex), and cytosol fractions of the all analyzed brain structures. In opposite to these data, in the 17-day-old rat pups prominent upregulation of the succinate dehydrogenase activities in the mitochondrial fraction of the all brain structures was noted with simultaneous their downregulation in the cytosol fractions of the orbital, limbic cortex and cerebellum. While looking at the activities of this enzyme in the brain structures of the 30-day-old rat pups, its significant upregulation was revealed mostly in the cytosol fractions of the all studied structures, though no changes of its activities were observed in the mitochondria fractions (except for the sensorimotor cortex).

DISCUSSION

The results of the studies indicate to existence of the phenomenon of transgenerational transmission (to the next generation) of the changes of the activities of pyrophosphatase and succinate dehydrogenase, induced originally by 10% hypoxia in the fetus of the rats at the stage of organogenesis. It should be emphasized that the results of the earlier studies showed that on the 17-day-old and 30-day-old rat pups, born after such prenatal exposure to hypoxia, the directions of the changes of the activities of these enzymes in the studied brain structures were similar to the characters of the changes of their activities in the brain structures of the rat pups of the same ages in our studies (next generation). In other words, on the 17-day-old and 30-day-old rat pups, born after the prenatal exposure to 10% hypoxia at the stage of organogenesis, downregulation of the activity of pyrophosphatase and upregulation of the activity of succinate dehydrogenase in the mitochondrial and cytosol fractions of the studied brain structures were observed.

These similarities of the characters of the changes of the activities of the said enzymes, apparently, indicate to transgenerational transmission of the genetic information of the changed genes activities. As changes of the sequences of nucleotides within a molecule of DNA under the impact of hypoxia (even at the stage of organogenesis) are unlikely to be considered as the basis for fixation of genetic information, the most probable mechanism of fixation of the observed changes in the enzymes activities on genetic level is the epigenetic changes of gene activities. The term ‘epigenetic’ refers to chromatin modifications which alter gene expression without affecting sequence of nucleotides within a molecule of DNA. The factors that promote the epigenetic regulation of transcriptional activity of the certain genes include microRNA, DNA methylation and posttranslational modifications (methylation, phosphorylation, acetylation and ubiquinilation) of histones of chromatin (Handy et al., 2011). Presently, there are mounting publications demonstrating successful transgenerational propagation of the various kinds of pathological states mediated through the epigenetic changes of genes’ activities obtained on different experimental models (Bohacek, Mansuy, 2013; Steenwyk et al., 2018).

In analyzing the obtained results on transgenerational transmission of the changes of activities of pyrophosphatase and succinate dehydrogenase, a reasonable question is raised: how specific the observed changes in the enzyme activities of the brain structures to the effects of prenatal hypoxia either these changes can be induced under the effects of any adverse factors? The certain changes of the activities of studied enzymes in the brain structures, especially upregulation of succinate dehydrogenase in the mitochondrial fraction of the brain structures of the 17-day-old rat pups and its upregulation in the cytosol fraction of the brain structures of the 30-day-old rat pups, could be considered as adaptive changes related directly to the effects of hypoxia to the fetus, due to important role for succinate dehydrogenase in the citric acid cycle and electron transport chain. It should be emphasized that footprints of the observed changes of the enzyme activities on the epigenome become possible due to the effect of hypoxia on the fetus on the period of organogenesis, for the most genes, being highly active in this period of embryogenesis, are extremely vulnerable to the effects of adverse conditions. Nevertheless, the valid conclusion on the specificity of hypoxia-in-

duced changes of the enzyme activities could be done only after studying the effects of other adverse factors, exposed in the same schedule to the fetus at the same stage of gestation, on the activities of these enzymes in the offspring.

REFERENCES

Abiyeva E.Sh. (2015) Impact of hypoxia, undergone in period of organogenesis, on dynamics of the activity of succinate dehydrogenase of the brain on the rats. *Proceedings of Azerbaijan NAS, biol. and med/ sci.*, **70(1):** 55-59.

Bohacek J., Mansuy I.M. (2013) Epigenetic inheritance of disease and disease risk. *Neuropsychopharmacology Reviews*, **38:** 220-236/

Handy D.E., Castro R., Loscalzo J. (2011) Epigenetic modifications: basic mechanisms and role in cardiovascular disease. *Circulation*, **123(19):** 2145–2156; doi: 10.1161/CIRCULATIONAHA.110.956839

Rashidova A.M., Babazadeh S.N., Mammedkhanova V.V., Abiyeva E.Sh. (2019) Dynamics of brain enzymes activity in rats exposed to hypoxia. *Biotechnologia Acta*, **12(4):** 42-49.

Steenwyk G., Roszkowski M., Manuella F., Franklin T.B., Mansuy I.M. (2018) Transgenerational inheritance of behavioral and metabolic effects of paternal exposure to traumatic stress in early postnatal life: evidence in the 4th generation. *Environmental Epigenetics*, **4(2):** 1-8; doi: 10.1093/eep/dvy023

Prenatal hipoksiyaya məruz qalmış sıçovul balalarının baş beyin strukturlarında iki enzimin dəyişilmiş fəallığının transgenerativ ötürülməsi

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Məqalə siçovullarda pirofosfataza və suksinatdehidrogenaza fermentlərinin fəallığının dəyişilməsinin transgenerativ ötürülməsinə həsr olunub. Təcrübələr Vistar xəttindən olan 6-aylıq dişi siçovullar və onlardan alınmış 17- və 30-günlük balalar üzərində aparılmışdır. Orqanogenez dövründə olan boğaz siçovullar 5 gün ərzində hər gün 20-dəqiqəlik hipoksiyaya məruz qalmışdırlar (90% N_2 və 10% O_2 qaz qarışığı). Bu siçovullardan alınmış balalar dekapitasiya olunmuş və onların baş beynindən orbital, hissi-hərəki, limbik qabıq, hipotalamus və beyincik götürülmüş və bunların mitoxondrial və sitozol fraksiyaları ayrılmışdır. Müəyyən olunmuşdur ki, 17-günlük siçovul balalarının bütün tədqiq olunan strukturların mitoxondrial fraksiyasında pirofosfataza fermentinin fəallığının mühüm azalması müşahidə edilmişdir, bucür dəyişiklik sitozol fraksiyasında yalnız orbital qabıqda müşahidə edilmişdir. 30-günlük siçovul balalarının baş beynin bütün strukturların mitoxondrial (orbital və limbik qabıq istisna olmaqla) və sitozol fraksiyasıyalarında pirofosfataza fermentinin fəallığının azalması qeydə alınmışdır. Bunun əksinə olaraq, 17-günlük siçovul balalarının bütün tədqiq olunan baş beynin strukturlarının mitoxondrial fraksiyasında suksinat dehidrogenazanın mühüm artımı müəyyən edilmişdir, eyni zamanda orbital, limbik qabıqların və beyinciyin sitozol fraksiyasında bu göstərici azalmışdır. 30-günlük sicovullarda bütün tədqiq olunan strukturların sitozol fraksiyasında bu fermentin fəallığının mühüm artımı qeyd olunmuşdur, bununla yanaşı mitoxondrial fraksiyasında gözə çarpan dəyişiklik aşkarlanmamışdır (hissi-hərəki qabıq istisna olmaqla). Belə nəticəyə gəlmək olar ki, hər iki fermentin fəallığının dəyişilməsinin transgenerativ otürülməsi, çox güman ki, müvafiq genlərin fəallığının epigenetik dəyişiklikləri ilə əlaqədardır.

Açar sözlər: *Pirofosfataza, suksinat dehidrogenaza, siçovul, baş beyin nahiyələri, transqenerativ ötürmə*

Трансгенерационная передача измененной активности двух ферментов в структурах головного мозга у потомства крыс, подвергнутых пренатальной гипоксии

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Статья затрагивает проблему трансгенерационной передачи изменённой активности ферментов пирофосфатазы и сукцината дегидрогеназы у крыс. Опыты были выполнены на 6-месячных крысах-самках линии Вистар и их потомстве в возрасте 17 и 30 дней. Беременные крысы, зародыши которых находились на стадии органогенеза, были подвергнуты 20-минутной ежедневной гипоксии (газовая смесь 90% азота и 10% кислорода) в течение 5 сут. Крысята, родившиеся от таких самок, были декапитированы и из их мозга были забраны фрагменты орбитальной, лимбической и сенсомоторной коры, гипоталамуса и мозжечка, из которых выделяли митохондриальную и цитозольную фракции. Было обнаружено, что у 17-дневных крысят наблюдалось заметное снижение активности пирофосфатазы в митохондриальной фракции всех изученных структур, тогда в цитозольной фракции активность пирофосфатазы снижалась только в орбитальной коре. В митохондриальной (кроме орбитальной и лимбической коры) и цитозольной фракциях всех структур 30-дневных крыс также наблюдалось снижение активности пирофорсфатазы. В противоположность этому у 17-дневных крысят в митохондриальной фракции всех исследованных структур отмечалось выраженное повышение активности сукцинат дегидрогеназы с одновременным её снижением в цитозольной фракции орбитальной, лимбической коры и мозжечка. У 30-дневных крысят значительное повышение активности этого фермента было выявлено в цитозольной фракции всех исследованных структур, в то время как в митохондриальной фракции не было выявлено каких-либо изменений (кроме сенсомоторной коры). Делается заключение о наличии трансгенерационной передачи изменённой активности двух ферментов, обусловленной, по-видимому, эпигенетическими изменениями в активности соответствующих генов.

Ключевые слова: *Пирофосфатаза, сукцинат дегидрогеназа, крысы, структуры головного мозга, трансгенерационная передача*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 30-37 (2020)

Tissue and subcellular activities of superoxide dismutase in skeletal muscles during physical exercises

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Accepted for publication: 22 May 2020

Activity of the enzyme superoxide dismutase (SOD) in the skeletal muscles was studied on the tissue and subcellular levels in the rats exposed to physical exercise. The specific differences in response to acute and regular training exercises were revealed in superoxide dismutase activities measured in tissue homogenate, mitochondrial and cytosolic fractions of white and red gastrocnemius muscles in the rats. These differences depended on exercise character (acute or chronic), fiber composition (glycolytic and oxidative) and subcellular localization of the enzyme. In the studied muscles, adaptive increase of SOD tissue activity was not shown in response to regular training exercise. In white muscles, preferentially composed of glycolytic fibers, adaptive induction of mitochondrial SOD (mitSOD) activity by regular training was more obvious than in red muscles, composed preferentially of oxidative fibers. However, induction of mitSOD activity by acute exercise in red muscles was displayed more strongly, than in white muscles. In red muscles of trained animals, the increase in SOD activity in the cytoplasm (cytSOD) becomes more moderate in response to testing exercise. In white muscles, cytSOD activity does not undergo adaptive changes and is not induced by testing exercise. Analysis of SOD activity in mitochondrial and cytoplasmic fractions of fast and slow muscles will be useful for elucidation of their adaptive peculiarities.

Keywords: *Skeletal muscles, physical exercise, superoxide dismutase, subcellular fractions*

INTRODUCTION

Aerobic organisms or their individual organs and tissues are able to adapt to the conditions of energy consumption. Physical exercise leads to a multiple increase in the demand of skeletal muscles for energy, which, in turn, is accompanied by increased pulmonary oxygen uptake. Here, however, muscle cells face a dangerous phenomenon for their structural and functional integrity - oxidative stress (Devies et al., 1982; Ji, 1999; Керимова и др., 2004; Powers et al., 2008; Steinbacher et al., 2015). Oxidative stress can occur when equilibrium between oxidants and antioxidants is disturbed. Oxidative stress occurs under conditions, when local antioxidant defenses are exhausted, because of oxidants or when the rate constants of the radical reactions are greater than the rate constants of the antioxidant defense mechanisms (Buettner, 1993; Vollaard et al., 2005). This could occur in skeletal muscles during acute exercise under conditions when oxidant/antioxidant balance shifts toward the pro-oxidant state.

It is well known that oxygen, which is the final electron acceptor in the respiratory chain of mitochondrias, plays a dual role in the life of cells; most of the oxygen entering the mitochondria, being completely reduced, turns into water, and a small part, according to various estimates, up to 5% of the total consumption, goes as a superoxide anion (O_2^-), which is a free radical (Halliwell et al., 1989; Halliwell, 2014; Скулачев, 1996). Aerobic cells, including muscle fibers, use superoxide dismutase (SOD) to fight O_2^- radicals, which promotes their dismutation and forms hydrogen peroxide (H_2O_2) and oxygen. Many

doi.org/10.29228/jlsb.39

works concern SOD as an important component of antioxidant protection of cells and tissues, among which the works, related to physical activity, occupy a significant place (Ji, 1999; Banerjee et al., 2003; Leeuwenburgh et al., 2011; Gomes et al., 2012).

In mammals, two isoforms of SOD exist in skeletal muscle; they vary in both cellular locations as well as in a metal ion bound to its active site. The Cu-Zn SOD is primarily located in the cytosol, whereas the Mn-SOD is principally found in the mitochondrial matrix (Ohno et al., 1994; Halliwell, 2014). Both enzymes catalyze the dismutation of superoxide anions with similar efficiency (Ohno et al., 1994). The Cu-Zn SOD is a dimer with a molecular weight of ~32,000 kDa, whereas the Mn SOD is a tetramer (MW = ~88,000 kDa) (Ohno et al., 1994).

It is well-established that the antioxidant defense systems of many mammalian tissues are capable of adaptation in response to chronic exposure to oxidants. Because prolonged physical exercise results in an increased production of oxidants in skeletal muscle, regular physical exercise training should bring to upregulation of muscle antioxidant enzyme systems. There is some evidence that endurance exercise training results in an increase in skeletal muscle antioxidant enzyme activity. Nonetheless, a few studies have failed to find augmented muscle antioxidant activity after physical exercise training. It can be assumed that these differences may be the result of differences in methodological approaches at choosing the types of muscles, fibers as well as the type of exercise training protocols (Powers et al., 1999). On the other hand, the existence of different isoforms of enzymes can also be the cause of conflicting statements concerning the role of an enzyme in the antioxidant response of muscles to physical activity (Laughlin et al., 1990; Tonkonogi et al., 2000; Hacıyev və b., 2013).

In this article, we present the results of the experiments on animals (albino rats) in studying the superoxide dismutase activity of skeletal muscles on the tissue and subcellular levels during acute exercise and regular physical training. The study was performed simultaneously over fast (white) and slow twitch (red) fiber type muscles of the same organism. Such approach makes it possible to analyze the activity of SOD in relation to the presence of various isoforms of the enzyme and their fiber affiliation to find out the antioxidant adaptive properties of skeletal muscles.

MATERIALS AND METHODS

Experiments were conducted on male Wistar albino rats of 250-300 g body mass kept in normal vivarium conditions. During the experiments, we followed the bioethical standards for the treatment of experimental animals in accordance with the European Convention for the protection of the rights of vertebrates used for experimental and scientific purposes (March 18, 1986, Strasbourg).

Rats were randomly pooled into four groups: untrained, non-exercised (*before removing the muscles*) (UN, n=6), untrained and exercised (UE; n=6), trained, non-exercised (TN n=6) and trained and exercised (TE, n=6).

The training process for the TN and the TE groups was carried out in a wheel with a diameter of 44 cm via running exercise. The load was given daily in the wheel rotation mode at a speed of 15-25 m/min, in the first days for 10-20 min, starting from the 3rd week the load duration was set to 30 min (~75% O_{2max}). Training process continued for 4 weeks, 5 days a week. Acute exercise (single physical load) was given by running in a wheel at a speed of 25 m/min for 30 min. Groups of sedentary animals that did not receive physical training loads (UN and UE) were subjected to running in a wheel for 10 minutes once a week for training in running under experimental conditions. A day after the end of physical training process, one group of the untrained (UE) and one group of the trained (TE) rats were subjected to an acute exercise, and immediately after that all animals were sacrificed and muscles were removed.

The calf muscle (*m.gastrocnemius*), its white and red components were studied. Red m. gastrocnemius (Type IIa fibers) is composed of highly oxidative fibers, but the white m. gastrocnemius (Type IIb fibers) is composed of highly glycolytic fibers. Mitochondria were obtained by centrifugation from 10% tissue homogenate in a sucrose medium (0.3M sucrose, 10 mM EDTA; pH 7.5). The activity of cytoplasmic SOD (cyt-SOD) was determined in the supernatant. The mitochondrial sediment was transferred to a phosphate buffer to

determine the activity of mitochondrial SOD (mit-SOD). The method for mitochondria isolation is described in (Прохорова, 1982).

The activity of SOD was determined by a method based on the competition of SOD with nitroblue tetrazolium in the reduction of superoxide radicals generated in the reaction of phenazine methosulphate (Дубинина, 1984).

The statistical reliability of differences between indicators of different groups was evaluated by the Student's t-criterion and the differences between average values were accepted as reliable at $p<0.05$.

RESULTS AND DISCUSSION

The results of study of SOD activity in skeletal muscle homogenate under the influence of regular training loads and acute single load are shown below (Table 1).

Table 1. Superoxide dismutase activity in the homogenate of skeletal muscle tissue in rats during exercise (arbitrary units/mg protein), $M \pm m$, $n=6$

Status / Muscles	Untrained		Trained	
	Rest (UN)	Exercise (UE)	Rest (TN)	Exercise (TE)
White	7.86±1.19	3.08±0.44*	8.17±1.22	6.18±0.93
Red	6.26±0.88	10.21±1.53*	6.96±0.90	9.98±1.35*

* $p<0.05$ compared the exercised groups with the termed "rest" groups;
# $p<0.05$ compared trained rest groups with untrained rest groups.

Four-week training loads do not lead to significant changes in SOD activity in the skeletal muscles at rest. However the decrease in SOD activity in the white muscle (white m. gastrocnemius), observed after the acute exercise in the untrained rats (UE), becomes less abrupt in the trained rats group (TE); that is, if the decrease in activity in the group UE relative to the group UN was 61% ($p<0.05$), then in the group TE relative to the group TU it became only 24% at $p>0.05$. In the red muscle (red m. gastrocnemius), the enzyme activity increases after the acute exercise by ~35% ($p<0.05$), both for the untrained and trained groups (UE vs.UN and TE vs. TN).

The results of studies of the effect of physical activity on superoxide dismutase activity in the subcellular fractions of skeletal muscles in the rats are presented in Table 2.

Under the influence of physical training, the changes in activity of SOD are observed, depending on both the fiber type of muscle and the subcellular affiliation of the enzyme. In the rats, that are not subjected to physical training (UN and UE groups), the activity of mit-SOD in the white muscle increases almost twice after acute physical exercise. However, in the red muscle, the activity of mit-SOD in the rats of these groups does not show significant changes in response to the same acute exercise, moreover, some statistically unreliable decrease ($p>0.05$) of activity is noted.

The baseline level (at rest) of mit-SOD activity in the white muscle increases by more than 2 times as a result of 4 weeks of physical training (the UN and the TN groups are compared). However, the reaction of mit-SOD activity in the trained rats to acute exercise (the TE vs. the TN) becomes different from the reaction of the untrained animals (the UE vs. the UN): the increase in activity under acute physical exercise in the trained rats disappears. There is only a tendency to decrease from rest activity of enzyme, resembling the situation with red muscle in the untrained rats.

In red muscle, the activity of mit-SOD does not change significantly under 4-week physical training. The 19% increase in activity (TN) compared to the untrained group (UN) of animals is statistically unreliable ($p>0.05$). As for the reaction of red muscle mit-SOD activity to acute exercise in the trained rats, here we observe a tendency to increase in contrast to the white muscle. Although the activity of mit-SOD of the red muscle in the trained rats after exercise (TE group) exceeds the baseline level for the TN group by 34% and the reliability of the difference is characterized by a low level of confidence ($p=0.06$), but it is noteworthy that there is a significant increase in the activity of mit-SOD in relation to the baseline activity of the untrained rats (UN, 0.205±0.025 arb.unit) ($p<0.05$).

Table 2. Superoxide dismutase activity in subcellular fractions of skeletal muscles in the rats during exercise (arbitrary units/mg protein). *M*±*m*, *n*=6

Status / Muscles	Untrained		Trained	
	Rest (UN)	Exercise(UE)	Rest (TN)	Exercise (TE)
Mitochondrial SOD				
White gastrocnemius	0.112±0.015	0.234±0.022*	0.307±0.031$^{\#}$	0.267±0.027$^{!}$
Red gastrocnemius	0.205±0.025	0.175±0.019	0.243±0.032	0.325±0.045$^{!}$
Cytoplasmic SOD				
White gastrocnemius	0.051±0.006	0.052±0.005	0.045±0.006	0.056±0.006
Red gastrocnemius	0.046±0.005	0.182±0.015**	0.043±0.005	0.102±0.012*$^{!}$

*, ** - $p<0.05$ and $p<0.01$ compared exercised groups with termed "rest" groups;
$^{\#}$ - $p<0.05$ compared trained rest groups with untrained rest groups;
$^{!}$ - $p<0.05$ compared trained exercised groups with untrained rest groups.

The changes in the activity of cyt-SOD in white and red muscles in response to acute exercise are different from the changes in the activity of the mitochondrial isoform. It should be noted that the rest levels of cys-SOD activity in the white and red muscle in the untrained rats (UN group) are almost equal, in contrast to mit-SOD, whose activity in the red muscle significantly exceeds the white muscle level.

After regular physical training for 4 weeks, the rest activity of cys-SOD of both muscles in the TN group rats remains at the level of activity for the rats in the UN group. Acute physical exercise does not lead to changes in the activity of cys-SOD in the white muscle in both the untrained (the UN and the UE groups) and the trained rats (the TN & the TE groups).

However, in the red muscles from the untrained and trained animals, SOD activity is greatly increased in the cytoplasm in response to exercise; the increase above baseline in the untrained rats is about 300% ($p<0.01$, compare UN and UE groups), and the trained somewhat lower, around 140% ($p<0.05$, compare TN and TE groups). We can say that in the red muscle, the reaction of superoxide dismutase activity in the cytoplasm, i.e., a sharp increase in the activity of the cyt-SOD in response to acute exercise becomes less abrupt as a result of regular physical training.

There are discrepant data on adaptive changes in SOD activity in skeletal muscles under the influence of physical training loads. Some studies indicate an increase in the activity of the enzyme after training (Higuchi et al., 1985; Leuwenburgh et al., 2001; Azizbeigi et al., 2014), while other studies show no significant changes in the activity of SOD (Alessio et al., 1988; Laughlin et al., 1990; Ji, 1993; Tonkonogi et al., 2000; White et al., 2017). These discrepancies could be explained by the fact that different authors' studies used different muscle types and fiber compositions, applied different exercise techniques, and, that is more important, measured the activity of different SOD isoforms. When our own data on measuring SOD activities at the tissue and subcellular levels are compared, there are also differences in responses to exercise.

If SOD activities of both types of muscles do not show significant changes at the tissue level with 4-week regular training loads, then subcellular activities, namely the activity of mit-SOD shows some adaptive growth, which is more significant in the white muscle. This result is consistent with the data of studies where it was concluded that cytosolic CuZn-SOD is passive in adapting to physical exertion in comparison with Mn-SOD (Higuchi et al., 1985; Ji, 1993; Lambertucci et al., 2007; Ristow et al., 2009).

A significant decrease in SOD tissue activity in white muscle after an acute exercise, which becomes moderate in the trained rats, seems paradoxical. However, if we pay attention to the changes in subcellular activity in response to an acute exercise, we can assume that the cytoplasmic component of SOD activity makes a greater contribution to the measured tissue activity. This assumption can be supported by the data from the work (Oh-Ishi et al., 1997), which shows an exercise-induced decrease in the level of m-RNA for CuZn-SOD in the untrained rats and the leveling of changes with regular training.

An increase in the red muscle tissue activity of SOD (both in the untrained and the trained rats) after a single acute exercise also indicates the pre-

valence of the proportion of cytoplasmic activity of the enzyme, which undergoes a 4-fold increase after acute exercise in the untrained rats and more than 2-fold increase in the trained ones. In particular, this is confirmed by the study in which it was shown that the activity of CuZn-SOD isoform of the enzyme in muscles increases significantly in response to acute exercise than the activity of the Mn-SOD isoform (Hollander et al., 1999; 2001; Lawler et al., 2009).

These results and a number of literature data on the content of protein and m-RNA of the SOD enzyme suggest that changes in the activity of mitochondrial and cytosolic isoenzymes in skeletal muscles may occur due to both post-transcriptional and post-translational effects of physical exercise (Hollander et al., 1999; 2001; Ji, 1999). In other words, the increased activity of SOD isoenzymes in skeletal muscles during exercise may be due to both the synthesis of a new protein and post-translational modification of a previously synthesized protein.

Our results indicate that the induction of SOD isoforms' activity by physical training is specific to the type of muscle fiber (see also, Hollander et al., 1999; 2001; Lawler et al., 1993). In white muscle, which consists primarily of a fast (glycolytic) type of fibers, adaptive upregulation of mit-SOD activity by training exercise is clearly visible. In the red muscle, which consists mainly of slow fibers, adaptive induction of mit-SOD activity by training exercise is weaker, however, the induction of activity by acute physical exercise is stronger than in the white muscle.

Differences of changes in SOD activity under the influence of regular training and acute physical exertion can probably be explained by differences in post-transcriptional and post-translational peculiarities of Mn-SOD and CuZn-SOD expression stimulated by physical exercise (Ji, 1999; Hollander et al., 1999; 2001). Taking into account the results of work (Oh-Ishi et al., 1997), which studied the activity of SOD isoforms, corresponding protein and m-RNA contents under the influence of physical exercise, we can assume that in fast type muscles, training loads lead to the induction of mitochondrial SOD activity by a post-transcriptional mechanism, and in slow type muscles - post-translational modulation of activity takes place. For cytoplasmic SOD, the effect of training loads is only seen in the slow-type muscles, and it is associated with the induction of activity due to post-translational changes in the protein.

Thus, we observe the participation of antioxidant protection of skeletal muscles in adaptive processes, associated with regular exercise at the level of enzymatic protection against superoxide anion radicals.

REFERENCES

Hacıyev Ə.M., Rzayev Z.B. (2013) Skelet əzələlərində antioksidant fermentlərin subhüceyrə aktivliyinin fiziki yükə reaksiyasının xüsusiyyətləri. Fiziologiya və biokimyanın problemləri. *AMEA A.İ.Qarayev ad. Fiziologiya institutu və Azərbaycan Fizioloqlar Cəmiyyə-tinin Elmi Əsərlərinin Külliyatı*. **31:** 94-102.

Дубинина Е.Е., Сальникова Л.А., Раменская Н.П., Ефимова Л.Ф. (1984) Перекисное окисление и антиокислительная система крови в онтогенезе. *Вопр. мед. Химии*, **5:** 28-32.

Керимова А.К., Гаджиев А.М. (2004) Влияние физических нагрузок на уровень перекисного окисления липидов в скелетных мышцах. *Актуальные проблемы физиологии и биохимии*, **30:** 256-264.

Скулачев В.П. (1996) Кислород в живой клетке: добро и зло. *Соросовский образовательный журнал*, **№3:** 2-10.

Прохорова М.И. (1982) Методы биохимических исследований. Изд-во С.-П. Унив., с. 29-43.

Alessio H.M., Goldfarb A.H. (1988) Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J. Appl. Physiol*., **64(4):** 1333-1336.

Azizbeigi K., Stannard S.R., Atashak S. et al. (2014) Antioxidant enzymes and oxidative stress adaptation to exercise training: Comparison of endurance, resistance, and concurrent training in untrained males. *Journal of Exercise Science & Fitness*, **12(1):** 1-6. https://doi.org/10.1016/j.jesf. 20 13.12.001

Banerjee A.K., Mandal A., Chanda D., Chakraborti S. (2003) Oxidant, antioxidant and physical exercise. *Molecular and Cellular Biochemistry*, **253(1-2):** 307-312.

Buettner G.R. (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch. Biochem. Biophys*., **300(2):** 535-543.

Davies K., Quintanilla A., Brooks G., Packer L. (1982) Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.*, **107(4):** 1198-1205.

Gomes E.C., Silva A.N., De Oliveira M.R. (2012) Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. Oxidative medicine and cellular longevity. *Hindawi Publishing Corporation*, **2012:** Article ID 756132, 12 p. DOI: 10.1155/2012/756132

Halliwell B. (2014) Cell culture, oxidative stress, and antioxidants: avoiding pitfalls. *Biomed. J.*, **37(3):** 99-105. doi: 10.4103/2319-4170.128725.

Halliwell B., Gutteridge J.M.C. (1989) Free radicals in Biology and Medicine (2nd ed). Oxford: Clarendon Press, pp.136-158

Higuchi M., Cartier L.J., Chen M., Holloszy J.O. (1985) Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. *J. Gerontol*., **40(3):** 281-286.

Hollander J., Fiebig R., Gore M. et al. (1999) Superoxide dismutase gene expression: fiber-specific adaptation to endurance training. *Am. J. Physiol*., **277(3):** R856-R862. DOI: 10.1152/ajp-regu.1999.277.3.R856

Hollander J., Fiebig R., Gore M. et al. (2001) Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pflugers Arch*., **442(3):** 426-34. DOI: 10.1007/s004240100539

Ji L.L. (1993) Antioxidant enzyme response to exercise and aging. *Med. Sci. Sports Exerc.*, **25(2):** 225-231.

Ji L.L. (1999) Antioxidants and oxidative stress in exercise. P*SEBM*, **222(3):** 283-292. DOI: 10.1046/j.1525-1373.1999.d01-145.x

Lambertucci R.H., Levada-Pires A.C., Rossoni L.V. et al. (2007**)** Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats. *Mech. Ageing Dev*., **128:** 267–275.

Laughlin M.H., Simpson T., Sexton W.L. et al. (1990) Skeletal muscle oxidative capacity, antioxidant enzymes, and exercise training. *J. Appl. Physiol*., **68(6):** 2337-2343. 10.1152/jappl. 1990.68.6.2337

Lawler J.M., Kwak H.B., Kim J.H. et al. (2009) Exercise training inducibility of MnSOD protein expression and activity is retained while reducing prooxidant signaling in the heart of senescent rats. *Am. J. Physiol. Regul. Integr. Comp Physiol*., **296(5):** R1496–R1502. doi: 10.1152/ajp regu.90314.2008

Leuwenburgh C., Hollander J., Leichtweis S. et al. (1997) Adaptations of glutathione antioxidant system to endurance training are tissue- and muscle fiber-specific. *Am. J. Physiol*., **272(1):** 363-369. DOI: 10.1152/ajpregu.1997.272.1.R363

Leeuwenburgh C., Heinecke J. W. (2001) Oxidative stress and antioxidants in exercise. *Current Medicinal Chemistry*, **8(7):** 829-838. DOI: 10.2174/0929867013372896

Oh-Ishi S., Kizaki T., Nagasawa J. et al. (1997) Effects of endurance training on superoxide dismutase activity, content, and mRNA expression in rat muscle. *Clin. Exp. Pharmacol. Physiol*., **24(5):** 326-332. 10.1111/j.1440-1681.1997.tb01196.x

Ohno H., Suzuki K., Fujii J. et al. (1994) Superoxide dismutases in exercise and disease. In: *Exercise and Oxygen Toxicity*, C.Sen, L.Packer, and O.Hanninen (Eds.). Amsterdam: Elsevier Publishers, p. 127-161.

Powers S.K.; Ji L.L, Leeuwenburgh K. (1999) Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. *Medicine & Science in Sports & Exercise*, **31(7)**: 987-997

Powers S.K., Jackson, M.J. (2008) Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. *Physiol. Rev*., **88(4)**: 1243–1276. DOI: 10.1152/physrev. 00031.2007

Ristow M., Zarse K., Oberbach A. et al. (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl. Acad. Sci. USA*, **106(21):** 8665–8670. doi: 10.1073/ pnas.0903485106

Steinbacher P., Eckl P. (2015) Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*, **5(2):** 356-377. doi:10.3390/ biom5020356

Tonkonogi M., Walsh B., Svensson M., Sahlin K. (2000) Mitochondrial function and antioxidative defence in human muscle: effects of endurance training and oxidative stress. *Journal of Physiology*, **528(2)**: 379-388. DOI: 10.1111/j.1469-7793.2000.00379.x

Vollaard N.B., Shearman J.P., Cooper C.E. (2005) Exercise-induced oxidative stress: Myths, realities and physiological relevance. *Sports Med.*, **35(12)**: 1045–1062. DOI: 10.2165/00007256-200535120-00004

White S.H., Warren L.K. (2017) Submaximal exercise training, more than dietary selenium supplementation, improves antioxidant status and ameliorates exercise-induced oxidative damage to skeletal muscle in young equine athletes *J. Anim. Sci*., **95(2):** 657-670. doi:10.2527/jas2016.1130

Fiziki yüklənmələr zamanı skelet əzələlərində superoksiddismutazanın toxuma və subhüceyrə aktivliyi

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Fiziki yükün təsiri altında ağ siçovulların skelet əzələlərində superoksiddismutaza (SOD) fermentinin aktivliyi toxuma və subhüceyrə səviyyələrində tədqiq edilmişdir. Ağ və qırmızı gastrocnemius əzələlərinin toxuma homogenatında, mitoxondri və sitozol subhüceyrə fraksiyalarında ölçülmüş SOD aktivliyinin kəskin və müntəzm məşq yüklərinə reaksiyalarında fərqlilik aşkar olunmuşdur. Bu fərqlər yükün xarakterindən (birdəfəlik, ya xroniki), əzələnin lif tərkibindən (qlikolitik, ya oksidativ) və fermentin subhüceyrə mənsubiyyətindən asılıdır. Tədqiq edilən əzələlərdə sakitlik halında SOD-un toxuma aktivliyində adaptiv yüksəlmə üzə çıxarılmayıb. Ağ əzələdə mitoxondrial SOD-un xroniki yüklə adaptiv induksiyası aşkar olunub. Qırmızı əzələdə mitoxondrial SOD-nın xroniki yüklə induksiyası zəifdir, ancaq onun kəskin yüklə induksiyası ağ əzələyə nisbətən özünü daha aydın göstərir. Məşqli heyvanlarda qırmızı əzələdə submaksimal fiziki yükə cavab olaraq SOD-un sitoplazma aktivliyinin yüksəlməsi mülayimləşir. Ağ əzələdə sitoplazma SOD aktivliyində məşq yükləri ilə adaptiv dəyişikliklər üzə çıxmır, testləşdirici fiziki yüklə induksiya da baş vermir. Müxtəlif əzələlərdə (sürət tipinə görə) superoksiddismutazanın subhüceyrə aktivliklərinin təhlili onların adaptasiya xüsusiyyətlərinin üzə çıxarılması üçün faydalı olacaq.

Açar sözlər: *Skelet əzələləri, fiziki yük, superoksiddismutaza, subhüceyrə fraksiyaları*

Тканевая и субклеточная активность супероксиддисмутазы скелетных мышц при физических нагрузках

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Изучалась активность супероксиддисмутазы в скелетных мышцах на тканевом и субклеточном уровнях под влиянием физических нагрузок у крыс. Обнаружены различия в изменениях активности СОД, измеренной в тканевом гомогенате, митохондриальной и цитоплазматической субфракциях белой и красной мышц *gastrocnemius,* в ответ на субмаксимальные и регулярные тренировочные нагрузки. Эти различия зависят от характера нагрузки (однократной или хронической), от волоконного состава (гликолитического или оксидативного) мышц, а также от субклеточной принадлежности фермента. В исследованных мышцах в состоянии покоя адаптивный рост активности СОД на тканевом уровне не обнаруживается. В результате регулярной тренировочной нагрузки имеет место адаптивная индукция активности митохондриальной СОД (мСОД) в белой (быстрой) мышце. В красной (медленной) мышце при хронической нагрузке адаптивная индукция активности м-СОД слабее, однако индукция активности острой физической нагрузкой (субмаксимальная нагрузка) проявляется сильнее, чем в белой мышце. В красной мышце тренированных животных увеличение активности СОД в цитоплазме (цСОД) в ответ на физическую нагрузку становится более умеренным. В белой мышце активность цСОД не обнаруживает адаптивных изменений и не индуцируется тестирующей физической нагрузкой. Анализ супероксиддисмутазной активности в субклеточных фракциях мышц различных типов может быть полезен для выявления их адаптационных свойств.

Ключевые слова: *Скелетные мышцы, физическая нагрузка, супероксиддисмутаза, субклеточные фракции*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 38-46 (2020)

The effects of heavy metals on biochemical processes in the human body (review)

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Accepted for publication: 05 June 2020

Environment and human health are closely related. Heavy metals are priority pollutants, as they are highly toxic to living organisms in relatively low concentrations. The World Health Organization considers them to be the most dangerous xenobiotics for human health. The paper reviews research on the toxic effects of heavy metals on biochemical processes in the human body and the clinical analysis of diseases caused by them. The author has presented the various mechanisms of heavy metal toxicity at the molecular level and shown the role of oxidative stress in the pathogenesis of diseases of the cardiovascular, nervous and respiratory systems, associated with the action of heavy metals, on a sufficiently large material. The analysis of literature data demonstrates the significant role and necessity of further study of biochemical mechanisms of the development of pathological processes under chronic exposure to various toxic heavy metals in order to develop effective methods of chronic diseases treatment.

Keywords: *Heavy metals, bioaccumulation, toxicity, biochemical disorders, complexing ability, oxidative stress*

Environmental pollution by toxic heavy metals is increasing worldwide and poses a growing threat to both the environment and human health. Intensive development of industry and chemicalization of agriculture led to accumulation of toxic for human organism chemical compounds in biosphere in large quantities. Today, due to the increasing anthropogenic load on the environment, the contamination of soil, water and wastewater with heavy metals is a worldwide problem (Теплая, 2013; Argos et al., 2010; Ogundiran et al., 2008). Dangerous levels of heavy metal pollution are observed in many industrialized areas of the world. (Гора, 2007; Дускаев и др., 2014; Теплая, 2013; Cheng, 2003).

Ecology and human health are closely interconnected. Heavy metals are priority pollutants, as they are highly toxic to living organisms in relatively low concentrations. Heavy metal ions do not disappear from the biological cycle from an ecotoxicological point of view, their toxicity does not decrease, but rather increases with increasing concentration. They possess high cumulative ability, therefore their danger lies in the possible long-term consequences (Рыбкин и др., 2014; Улахович и др., 2010; Фрумин, 2002; Batariova et al., 2006; Cheng, 2003).

It was found out that metals and their compounds accumulate in soil relatively quickly, are persistent and are removed from it very slowly (Мудрый, 2008). A lot of research is devoted to the study of heavy metals in soil. Sources of heavy metals entering the soil are examined in detail, and the content of a number of metals is analyzed. It is shown that the main anthropogenic sources of metals are various fuel installations, ferrous and non-ferrous metallurgy enterprises, mining enterprises, cement plants, chemical enterprises, galvanic industries and transport. The sources of heavy metals in soil are considered in detail and the content of a number of metals is analyzed. The heavy metals accumulated in soil and water undergo biomagnification in the food chain and then bioaccumulate in living organisms (Дускаев и др., 2014; Chiocchetti et al., 2017; Ogundiran et al., 2008; Rai et al., 2003).

According to scientific studies, the special place of metals among the priority chemicals polluting

doi.org/10.29228/jlsb.40

the biosphere is due to the following reasons (Батян и др., 2009; Фрумин, 2002):

• The rate of extraction of metals from the Earth's crust by humans is higher than the geological rate of their extraction (Table 1).

• Unlike organic pollutants undergoing decomposition processes, metals are capable of redistribution between the individual components of the geographic envelope.

Table 1. Recovery rate of metals from the Earth's crust (tons / year)

Element	«Geological speed» Vg	extraction rate by Humans Vh	Vh/Vg
Iron	$2{,}5 \cdot 10^7$	$3{,}2 \cdot 10^8$	12,8
Copper	$3{,}8 \cdot 10^5$	$4{,}5 \cdot 10^6$	11,8
Zinc	$3{,}7 \cdot 10^5$	$3{,}9 \cdot 10^6$	10,5
Lead	$1{,}8 \cdot 10^5$	$2{,}3 \cdot 10^6$	12,7
Manganese	$4{,}4 \cdot 10^5$	$1{,}6 \cdot 10^6$	3,6
Tin	$1{,}5 \cdot 10^3$	$1{,}7 \cdot 10^5$	113
Molybdenum	$1{,}3 \cdot 10^4$	$5{,}7 \cdot 10^4$	4,4
Mercury	$3{,}0 \cdot 10^3$	$7{,}0 \cdot 10^3$	2,3
Silver	$5{,}0 \cdot 10^3$	$7{,}0 \cdot 10^3$	1,4

Metals are relatively easy to accumulate in the soil, but are difficult and slow to remove from it. Half-removal period of zinc is up to 500 years, cadmium - up to 1100 years, copper - up to 1500 years, lead - up to several thousand years.

• Metals are well accumulated by human organs and tissues, warm-blooded animals and aquatic animals.

• Metals, especially heavy metals, are highly toxic for various biological objects.

More than 40 metals of D.I. Mendeleev's periodic table of elements with atomic mass of more than 50 atomic units are considered to be heavy metals (Куценко, 2004; Лысенко и др., 2015; Фрумин, 2002). In terms of their toxicity, prevalence and ability to accumulate in food chains, 10 elements are recognized as priority pollutants of the biosphere and are subject to priority control. These are Pb, Cu, Zn, Ni, Cd, Co, Sb, Sn, Bi, Hg. The maximum permissible concentrations of trace elements in the human body are established (Table 3).

The amplitude of the content of an element in different organisms may significantly exceed the specified concentrations. The concentration factor has a determining character for evaluation of the physiological action of the element. It is known that each element has an inherent safe exposure range that supports optimal tissue concentrations and functions. Each element has its own toxic range when its safe exposure is exceeded (Батян и др., 2009; Куценко, 2004; Лысенко и др., 2015; Фрумин, 2002). To assess the degree of hazard of heavy metals in toxicological chemistry, according the Mertz rule all metals are divided into three groups (the smaller the range, the "more dangerous"): (I) As, Be, Cd, Hg, Pb, Tl, Zn; (II) B, Co, Cr, Cu, Mo, Ni, Sb, Sc; (III) Ba, Mn, Sr, V, W.

It is generally recognized that the most dangerous elements for humans, and indeed for warm-blooded animals, are cadmium, mercury and lead (Оберлис и др., 2008; Полина, 2012; Улахович и др., 2010; Фрумин, 2002; Bjermo et al., 2013; Martinez et al., 2011). A chemical element is considered vital if, in its absence or inadequate intake into the body, normal vital activity is disrupted, development stops. As a result of exposure to toxic elements, intoxication syndrome develops in the body. Each element has its own operating concentration range, which allows vital functions to be performed. If there is a deficiency or excess of an element, the work of the enzymes that are dependent on them suffers first. Homeostasis of metals and ligands is disturbed, pathological changes develop.

Heavy metals are considered by the World Health Organization as the most dangerous to human health xenobiotics. Daily prolonged exposure to contaminated food and water leads to the accumulation of metals in the human body and the development of various severe diseases (Jomova et al., 2011; Kyrre et al., 2017; Martinez et al., 2011; Young-Seoub et al., 2014). Heavy metal pollution more often is recognized as dramatic in many countries of the developing world. There are a significant number of large-scale studies in various countries to determine the content of heavy metals in the environment and biological fluids and human tissues to assess toxicity (Batariova et al., 2006; Bibi et al., 2015; Bjermo et al., 2013; Cheng, 2003; Forte et al., 2011).

It has been shown that the main sources of exposure to heavy metals are food, water and airborne particulates, including smoke (Улимбашев и др., 2012; Hyun-Jun et al., 2017; Kyrre et al., 2017; Rahmani et al., 2018).

Table 2. Toxic effects of heavy metals and the diseases caused by them

Metal	Acute	Chronic	Toxic Concentration
Arsenic	Nausea, vomiting, "rice-water" diarrhea, encephalopathy, MODS, LoQTS, painful neuropathy	Diabetes, hypopigmentation/ hyperkeratosis, cancer: lung, bladder, skin, encephalopathy	24-h urine: ≥50 μg/L urine, or 100 μg/g creatinine
Bismuth	Renal failure; acute tubular necrosis	Diffuse myoclonic encephalopathy	No clear reference standard
Cadmium	Pneumonitis (oxide fumes)	Proteinuria, lung cancer, osteomalacia	Proteinuria and/or ≥15 μg/ g creatinine
Chromium	GI hemorrhage, hemolysis, acute renal failure (Cr^{6+} ingestion)	Pulmonary fibrosis, lung cancer (inhalation)	No clear reference standard
Cobalt	Beer drinker's (dilated) cardiomyopathy	Pneumoconiosis (inhaled); goiter	Normal excretion: 0.1-1.2 μg/L (serum) 0.1-2.2 μg/L (urine)
Copper	Blue vomitus, GI irritation/ hemorrhage, hemolysis, MODS (ingested); MFF (inhaled)	Vineyard sprayer's lung (inhaled); Wilson disease (hepatic and basal ganglia degeneration)	Normal excretion: 25 μg/24 h (urine)
Iron	Vomiting, GI hemorrhage, cardiac depression, metabolic acidosis	Hepatic cirrhosis	Nontoxic: < 300 μg/dL Severe: >500 μg/dL
Lead	Nausea, vomiting, encephalopathy (headache, seizures, ataxia, obtundation)	Encephalopathy, anemia, abdominal pain, nephropathy, foot-drop/ wrist-drop	Pediatric: symptoms or [Pb] ≥45 μ/dL (blood); Adult: symptoms or [Pb] ≥70 μ/dL
Manganese	MFF (inhaled)	Parkinson-like syndrome, respiratory, neuropsychiatric	No clear reference standard
Mercury	Elemental (inhaled): fever, vomiting, diarrhea, ALI; Inorganic salts (ingestion): caustic gastroenteritis	Nausea, metallic taste, gingivo-stomatitis, tremor, neurasthenia, nephrotic syndrome; hypersensitivity (Pink disease)	Background exposure "normal" limits: 10 μg/L (whole blood); 20 μg/L (24-h urine)
Nickel	Dermatitis; nickel carbonyl: myocarditis, ALI, encephalopathy	Occupational (inhaled): pulmonary fibrosis, reduced sperm count, nasopharyngeal tumors	Excessive exposure: ≥8 μg/L (blood) Severe poisoning: ≥500 μg/L (8-h urine)
Selenium	Caustic burns, pneumonitis, hypotension	Brittle hair and nails, red skin, paresthesia, hemiplegia	Mild toxicity: [Se] >1 mg/L (serum); Serious: >2 mg/L
Silver	Very high doses: hemorrhage, bone marrow suppression, pulmonary edema, hepatorenal necrosis	Argyria: blue-grey discoloration of skin, nails, mucosae	Asymptomatic workers have mean [Ag] of 11 μg/L (serum) and 2.6 μg/L (spot urine)
Thallium	Early: Vomiting, diarrhea, painful neuropathy, coma, autonomic instability, MODS	Late findings: Alopecia, Mees lines, residual neurologic symptoms	Toxic: >3 μg/L (blood)
Zinc	MFF (oxide fumes); vomiting, diarrhea, abdominal pain (ingestion)	Copper deficiency: anemia, neurologic degeneration, osteoporosis	Normal range: 0.6-1.1 mg/L (plasma) 10-14 mg/L (red cells)

MODS- multi-organ dysfunction syndrome; MFF- metal fume fever; GI-gastrointestinal; LoQTS-long QT syndrome and a rare inborn heart condition; ALI-acute lung injury.

The most dangerous non-degradable elements, toxic even in trace amounts, according to the FAO/ WHO Food Codex Commission (Codex Alimentarius), are Hg, Cd, Pb, Sn, V, Mo, As, Co (www.fao.org/fao-who-codexalimentarius).

Health problems due to environmental pollution by heavy metals are becoming a serious problem today in the world and are widely studied. Heavy metals entering the human body violate the regulation of many physiological functions, biochemical and morphological disorders are identified (Jomova et al., 2011; Young-Seoub et al., 2014). They disrupt metabolic processes (Planchart et al., 2018) have a toxic effect on many systems of the human body, including the nervous (Jomova et al., 2010), immune (Балабекова и др.,

2015; Tasleem et al., 2015) and cardiovascular systems (Prozialeck et al., 2008), kidneys (Reyes et.al., 2013) and other.

Table 3. Maximum permissible concentrations of trace elements in the human body.

Element	Blood (mkg/ml)	Urine (mkg/ml)
Mn (Manganese)	0,06	0,07
Ag (silver)	0,1	0,06
As (arsenic)	0,2	0,004
Va (barium)	0,08	0,8
Cd (cadmium)	0,005	0,04
Bi (bismuth)	0,03	0,02
Cr (chrome)	0,004	0,02
Cu (copper)	0,9	0,1
Pb (lead)	0,25	0,08
Tl (Thallium)	0,01	0,002
Zn (zinc)	1,2	1,2

The physiological and biochemical effect of heavy metals on the human body is different and depends on the nature of the metal, the type of compound in which it exists in the natural environment, as well as its concentration. Not all heavy metals are equally dangerous to human health. According to a classification that takes into account the physiological role, chemical elements are divided into (Куценко, 2004; Оберлис и др., 2008):

- essential: Fe, I, Cu, Zn, Co, Cr, I, Mo, Se, Mn;
- conditionally essential: As, B, Br, F, Li, Ni, Si;
- toxic: Al, Cd, Pb, Hg, Be, Ba, Bi, Tl;
- potentially toxic: Ag, Au, In, Ge, Rb, Ti, Te, U, W, Sn, Zr.

Among heavy metals, some are essential for the vital functions of man and other living organisms. They are referred to as essential nutrients and are called trace elements (Оберлис и др., 2008)). They participate in the biochemical processes of the human body and are necessary for its normal functioning. Such metals include iron, zinc, molybdenum, copper, etc. However, in large quantities, these metals and their compounds are toxic and can have harmful effects on the body. It should be remembered that each trace element has its own working range of concentrations, which allows it to perform vital functions. Deficiency or excess of an element leads at first to disturbance the activity of the enzymes in which they are included. The homeostasis of metals and biological molecules is disrupted, pathological changes develop.

Other heavy metals are not used by the body, are highly toxic and can accumulate in tissues, leading to poisoning or even death (Батян и др., 2009; Дускаев др., 2014; Лысенко и др., 2015; Оберлис и др., 2008). These metals are called toxic and belong to the class of xenobiotics, that is alien to living organisms and are considered systemic toxicants. The toxicity of “metallic poisons” is explained by their binding to the relevant functional groups of protein and other vital compounds in the body. As a result, the normal functions of the relevant cells and tissues in the body are disrupted. Clinical symptoms vary depending on the metal, its dose and whether the exposure was acute or chronic (Table 2).

Although toxicity resulting from exposure to significant amounts of toxic metals usually affects many organ systems, the severity of the health consequences depends on the type of chemical structure and element shape, the route and duration of exposure, and, to a greater extent, depends on the individual's susceptibility (Батян и др., 2009; Полина, 2012). For example, barium sulfate is generally non-toxic, however barium is rapidly absorbed and cause deep, potentially fatal hypokalemia. Mercury is relatively inert in the gastrointestinal tract and is also poorly absorbed through intact skin, but inhalation or injection of mercury can be disastrous.

Age also influences toxicity. For example, younger children are more susceptible to heavy metals, and even short-lived effects can affect the child's development processes (Blaurock-Busch et al., 2011; Molina-Villalba et al., 2015). Experimental studies have shown that blood concentrations of Pb, As, Cd and Mn in animals varied significantly between young and older age groups. All components except Mn were higher in older age groups (Тухватшин и др., 2017).

Chronic poisoning due heavy metal of lead acetate and potassium dichromate in experimental animals disrupts carbohydrate and fat metabolism, as well as changes in protein metabolism and the enzyme system in animals, especially older ones, in which recovery processes are less pronounced (Балабекова и др., 2015; Тухватшин и др., 2017). It was found that the course of aseptic inflammation under conditions of preliminary poisoning of rats with heavy metal compounds without treatment is aggravated by a tendency to the chronization of the process (Тухватшин и др., 2017).

Heavy metals are characterized by high permeability and the ability to be absorbed into the blood and after spread to organs, tissues and settling in them. The liver and kidneys, which filtering out toxins and removing them, take the main blow. If these organs suffer greatly, their functions were violated - from this moment the removal of metal compounds from the body is impossible. As a result, intoxication does not decrease, and toxins without interference affect the nervous, cardiovascular and respiratory systems.

The cardiovascular system, also the kidneys, is one of the first to be exposed to the toxic effects of xenobiotics. There is an increase in cardiovascular and renal pathology in the population in ecologically unfavorable areas. In addition, as a result of detailed studies conducted over the past decade, it has been revealed that heavy metals play a role in the emergence and development of such widespread diseases as hypertension, diabetes mellitus, and a number of neurological and oncological diseases (Jomova et al., 2010; Sharma et al., 2006; Waisberg et al., 2003).

The toxicity of heavy metals at the molecular level has three mechanisms. The first mechanism is determined by the ability of heavy metals to bind functional groups of biologically important substances in the body, primarily to block sulfhydrilic groups of SH-enzymes (Куценко, 2004; Forte et al., 2011; Winterbourn et al**.,** 2008). As a result of the reaction of metal ions with SH-groups of enzymes, are formed weakly dissociating and insoluble compounds - mercaptides. The formation of mercaptides is accompanied by damage to proteins, a violation of their function, which initiates the development of a toxic process. Damage to intra-protein bonds leads to their denaturation, thereby changing their main functions - transport, structural and enzymatic. In this case, thiol poisons and their compounds interact with SH-groups of amino acids that form proteins with carboxyl groups.

The second mechanism of the toxic effect of heavy metals is based on the displacement of biogenic metals from metal-containing complexes (Jacobson et al., 2012; Sharma et al., 2011; Tamas et al., 2014). If the stability of a metal-containing complex is greater than that of biogenic metals, the equilibrium shifts to the right and toxic metals accumulate in the body, which leads to disruption of normal body function. This mechanism is due to the proximity of the geometric dimensions and charges of ions of biogenic and toxic metals. It should be noted that strength of chemical bonds of proteins and other biologically important components of blood with ions of any metal is sufficient for considerable part of time of its stay in organism metal was in the form of complex with proteins, amino acids and other biologically active compounds. Therefore, if an excess of metals enters the body, the latter can cause a violation of its functions, poisoning or death. The degree of such influence depends not only on the concentration exceeding a certain level, but also on the nature of the metal, primarily its complexing ability. Thus, if the complex-forming ability of the metal-toxicant is large enough, it can displace the biogenic metal-catalyst from the active center as a result of competitive interaction or bind with itself the overwhelming part of biologically active compounds used for the synthesis of this or that vital enzyme. Heavy metals can cause disruption in cellular processes by displacing irreplaceable metals from their respective locations. Oxidative degradation of biological macromolecules has been found to be mainly associated with metal binding to DNA and nuclear proteins (Реутова, 2015; Abilev et al., 2013; Flora et al., 2008; Ghosh et al., 2012).

The third mechanism is caused by the development of oxidative stress under the influence of heavy metals (Cuypers et al., 2010; Ercal et al., 2001; Jacobson et al., 2012; Leonardo et al., 2017; Sharma et al., 2014). Redox active metals such as iron, copper and chromium, as active redox agents, directly enhance redox reactions, while redox inactive metals such as lead and cadmium, mercury and others deplete the main antioxidants in cells, especially thiol-containing antioxidants and enzymes, while cadmium, arsenic and lead exhibit their toxic effects by binding to sulfhydride protein groups and depleting glutathione (Jomova et al., 2011). Silver nanoparticles have been shown to induce oxidative stress and chromosome aberrations (Ghosh et al., 2012).

It is believed that the factor of metal toxicity and carcinogenicity is the formation of reactive oxygen species that cause lipid peroxidation, membrane destruction and damage to proteins, carbohydrates and DNA.

It has been established that chronic exposure to metals leads to protein damage, which contributes to the progression of neurodegenerative diseases. A detailed understanding of the oxidative damage to proteins caused by metal is provided by Reyes et al (2013) and Tamas et al. (2014) and others (Ghezzi, 2005; Sharma et al., 2014; Valko et al., 2006; Winterbourn et al., 2008). Thus, the analysis of literature data shows the significant role and necessity of further study of biochemical mechanisms of pathological processes development under chronic exposure of various toxic heavy metals in order to develop effective methods of chronic diseases treatment.

REFERENCES

Багандова Л. М., Астарханова Т. С., Ашурбекова Т. Н. (2011) Биоэкологический мониторинг антропогенных воздействий при разных видах хозяйственной деятельности. *Юг России: экология, развитие*, **6 (3):** 99-101.

Балабекова М.К., Рыспекова Н.Н., Жукешева М.К. и др. (2015) Динамика течения воспаления, вызванного на фоне металлиндуцированной иммунодепрессии. *Ж. Современные проблемы науки и образования*, **6:** 276 с.

Батян А. Н., Фрумин Г. Т., Базылев В. Н. (2009) Основы общей и экологической токсикологии. СПб.: СпецЛит., 352 с.

Гора Е.П. (2007) Экология человека. Учебное пособие. М., 540 с.

Дускаев Г. К., Мирошников С. А., Сизова Е. А. и др. (2014) Влияние тяжелых металлов на организм животных и окружающую среду обитания (обзор). *Вестник мясного скотоводства*, **3:** 7-11.

Куценко С.А. (2004) Основы токсикологии. М.: Фолиант, 570 с.

Лысенко Н. Н., Догадина М. А. (2015) Основы экотоксикологии: учеб. пособие. Орел ГАУ, 460 с.

Мудрый И.В. (2008) Влияние химического загрязнения почвы на здоровье населения. *Гигиена и санитария*, **4:** 32-37.

Оберлис Д., Харланд Б., Скальный А. (2008) Биологическая роль макро- и микроэлементов у человека и животных. СПб.: Наука, 544 с.

Полина И. Н. (2012) Токсикология: учебное пособие. Сыктывкар: СЛИ, 2012: 128 с.

Ревич Б.А. (2007) Проблемы прогнозирования, «горячие точки» химического загрязнения окружающей среды и здоровье населения России. Под ред. В.М.Захарова. Москва, 192 с.

Реутова Н. В. (2015) Мутагенный потенциал ряда тяжелых металлов. *Экологическая генетика,* **13 (3):** 70-75.

Рыбкин В.С., Богданов А.Н., Чуйков Ю.С., Теплая Г.А. (2014) Тяжелые металлы как фактор возможных экологически обусловленных заболеваний в астраханском регионе. *Гигиена и санитария*, **2:** 27-31.

Теплая Г. А. (2013) Тяжелые металлы как фактор загрязнения окружающей среды. *Астраханский вестник экологического образования*, **1 (23):** 182-192.

Тухватшин Р.Р., Аумолдаева З.М., Абаева Т.С., Исупова А.А. (2017) Биохимические показатели крови у животных различного возраста при отравлении тяжелыми металлами. *Патологическая физиология*, **1 (35):** 39-44 .

Улахович Н.А., Кутырева М.П., Медянцева Э.П., Бабкина С.С. (2010) Экотоксиканты : учеб.-метод. пособие для лекционного курса «Химия в экологии». Казань: Казанский Государственный Университет, 56 с.

Улимбашев М. Б., Тхашигугова М. С. (2012) Концентрация тяжелых металлов в сельскохозяйственной продукции, полученной в различных экологических зонах. *Достижения вузовской науки*, **1:** 110-114.

Фрумин Г.Т. (2002) Экологическая химия и экологическая токсикология: учеб. пос. СПб.: РГГМУ, 107-111.

Abilev S., Glazer V. (2013) Geneticheskaja toksikologija: itogi i problemy. [Genetic toxicology: results and problems]. *Russian journal of gene*, **49 (1):** 81-93.

Argos M., Kalra T., Rathouz P. et al. (2010) Arsenic exposure from drinking water, and all-cause and chronic-disease mortalities in Bangladesh (HEALS): a prospective cohort study. *Lancet*, 376 **(9737):** 252-258.

Batariova A., Spevackova V., Benes B. et al. (2006) Blood and urine levels of Pb, Cd and Hg

in the general population of the Czech Republic and proposed reference values. *Int. J. Hyg. Environ. Health*, **209:** 359-366.

Bibi M., Hashmi M., Malik R. (2015).The level and distribution of heavy metals and changes in oxidative stress indices in humans from Lahore district, Pakistan. *Hum. Exp. Toxicol*., **35 (1):** 78-90.

Blaurock-Busch E., Amin R. O., Rabah T. (2011) Heavy Metals and trace elements in hair and urine of a sample of Arab children with autistic spectrum. *Disorder Maedica (Buchar.)*, **6(4):** 247-257.

Bjermo H., Sand S., Nalsen C. et al. (2013) Lead, mercury, and cadmium in blood and their relation to diet among Swedish adults. *Food Chem. Toxicol*., 57**:** 161-169.

Cheng S. (2003) Heavy metal pollution in China: Origion, pattern and control. *Environ. Sci. Pollut. Res. Int*., **10:** 192–198.

Chiocchetti G., Jadán-Piedra C., Vélez D., Devesa V. (2017) Metal (loid) contamination in seafood products. *Crit. Rev. Food Sci. Nutr.,* **57 (17):** 3715-3728.

Cuypers A., Plusquin M., Remans T. et al. (2010) Cadmium stress: an oxidative challenge. *Biometals*, **23:** 927-940.

Ercal N., Gurer-Orhan H., Aykin-Burns N. (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem*., **1(6):** 529-539.

Flora S., Mittal M., Mehta A. (2008) Heavy metal induced oxidative stress and its reversal by chelation therapy. *Ind. J. Med. Res*., **128:** 501-523.

Forte G., Madeddu R., Tolu P. et al. (2011) Reference intervals for blood Cd and Pb in the general population of Sardinia (Italy). *Int. J. Hyg. Environ. Health*, **214:** 102-109.

Ghezzi P. (2005) Oxidoreduction of protein thiols in redox regulation. *Biochem. Soc. Trans*., **33:** 1378-1381.

Ghosh M., Manivannan J., Sinha S. et al. (2012) In vitro and in vivo genotoxicity of silver nanoparticles. *Mutat. Res*., **749 (1-2):** 60-69.

http://www.fao.org/fao-who-codexalimentarius/about-codex/ru/#c453333

Hyun-Jun K., Hwan-Sub L., Kyoung-Ryul L. (2017) Determination of trace metal levels in the general population of Korea. *Int. J. Environ. Res. Public Health*, **14(7):** 702 p.

Jacobson T., Navarrete C., Sharma S.K. et al. (2012) Arsenite interferes with protein folding and triggers formation of protein aggregates in yeast. J. Cell Sci. **125 (Pt. 1):** 5073-5083.

Jomova K., Valko M. (2011) Advances in metal-induced oxidative stress and human disease. *Toxicology*, **283(2-3):** 65-87

Jomova K., Vondrakova D., Lawson M. (2010) Metals, oxidative stress and neurodegenerative disorders. Mol. Cell Biochem. **345 (1-2):** 91-104.

Kyrre S., Pacyna J., Elisabeth G. et al. (2017) Thorne Global Sources and Pathways of Mercury in the Context of Human Health. *Int. J. Environ Res Public Health*., **14(1):** 105.

Leonardo O. Bittencourt B., Senda C. et al. (2017) Oxidative biochemistry disbalance and changes on proteomic profile in salivary glands of rats induced by chronic exposure to methyl mercury. *Oxid. Med. Cell Longev*., **2017:** 5653291.

Martinez V., Vucic E., Becker-Santos D. et al. (2011) arsenic exposure and the induction of human cancers. *J. Toxicol*., **2011:** 431-487.

Molina-Villalba I., Lacasaña M., Rodríguez-Barranco M. et al. (2015) Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. *Chemosphere*, **124:** 83-91.

Ogundiran M., Osibanjo O. (2008) Heavy metal concentration in soils and accumulation in plants growing in a deserted slag dump site in Nigeria. *Afr. J. Biotechnol*., **7:** 3053-3060.

Planchart A., Green A., Hoyo C., Mattingly C. (2018) Heavy metal exposure and metabolic syndrome: Evidence from human and model system studies. *Curr. Environ. Health Rep*., **5(1):** 110-124.

Rai U., Tripathi R., Vajpayee P. et al. (2003) Cadmium accumulation and its phytoxicity in *Potamogeton pectinatus* L. (*Potamogetona-ceae*). *Bull. Environ. Contam. Toxicol*., **70:** 566-575.

Prozialeck W., Edwards J., Nebert D. et al. (2008) The vascular system as a target of metal toxicity. *Toxicol. Sci*., **102:** 207-218.

Rahmani J., Fakhri Y., Shahsavani A. et al. (2018) A systematic review and meta-analysis

of metal concentrations in canned tuna fish in Iran and human health risk assessment. *Food Chem. Toxicol*.. **118:** 753-765.

Reyes J., Molina-Jijon E., Rodriguez-Munoz R. et.al (2013) Tight junction proteins and oxidative stress in heavy metals induced nephrotoxicity. *BioMed. Res. Int*., **2013:** 730-789.

Sharma A., Talukder G. (1987) Effects of metals on chromosomes of higher organisms. *Environ. Mutagenesis*, **9:** 191-226.

Sharma B., Singh S., Siddiqi N. (2014) Biomedical implications of heavy metals induced imbalances in redox systems. *Biomed. Res. In*t., **2014:** 640-654.

Sharma S., Goloubinoff P., Christen P. (2008) Heavy metal ions are potent inhibitors of protein folding. *Biochem. Biophys. Res. Commun*., **372:** 341-345.

Sharma S., Goloubinoff P., Christen P. (2011) Non-native proteins as newly identified targets of heavy metals and metalloids. In: *G.Bánfalvi (ed.) Cellular Effects of Heavy Metals*. Germany, Heidelberg: Springer, p. 263-274.

Tamas M., Sharma S., Ibstedt S. et al. (2014) Heavy metals and metalloids as a cause for protein misfolding and aggregation. *Biomolecules*, **4:** 252-267.

Tasleem A. Azam M., Siddiqui K. et al. (2015) Heavy metals and human health: Mechanistic insight into toxicity and counter defense system of antioxidants. *Int. J. Mol. Sci*., **16 (12):** 2959-2963.

Valko M., Rhodes C.J., Moncol J., Izakovic M., Mazur M. (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact*., **160 (1):** 1-40.

Valko M, Jomova K, Rhodes C. et al. (2016) Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. *Arch. Toxicol*., **90 (1):** 1-37.

Waisberg M., Joseph P., Hale B., Beyersmann D. (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, **192:** 95-117.

Winterbourn C., Hampton M. (2008) Thiol chemistry and specificity in redox signalling. *Free Radic. Biol. Med*., **45:** 549-561.

Young-Seoub H., Ki-Hoon S., Jin-Yong C. (2014) Health effects of chronic arsenic exposure. *J. Prev. Med. Public Health*, **47(5):** 245-252.

Ağır metallar və onların insan orqanizmində biokimyəvi proseslərə təsiri

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Ekologiya və insan sağlamlığı biri-biri ilə sıx bağlıdır. Ağır metallar ətraf mühit çirkləndiriciləri arasında canlı orqanizmlər üçün ən zərərlilərdəndir, çünki onlar nisbətən aşağı konsentrasiyalarda öz toksiki təsirini göstərir. Ağır metallar Ümumdünya Səhiyyə Təşkilatı tərəfindən insan sağlamlığı üçün ən təhlükəli ksenobiotik kimi qəbul edilmişdir. Bu məqalə ağır metalların orqanizmdə biokimyəvi proseslərə təsiri və onların yaratdığı xəstəliklərin kliniki təhlili üzrə aparılan tədqiqatların icmalına həsr olunmuşdur. Ağır metalların toksikliyinin müxtəlif molekulyar mexanizmləri təqdim edilmişdir və kifayət qədər böyük materialda ağır metalların təsiri ilə bağlı ürək-damar, sinir və tənəffüs sistemləri xəstəliklərinin patogenezində oksidləşdirici stresin rolu müzakirə edilmişdir. Ədəbiyyat məlumatlarının təhlili göstərir ki, müxtəlif toksiki ağır metalların xroniki təsiri zamanı patoloji proseslərin inkişafının biokimyəvi mexanizmlərinin öyrənilməsi və gələcək araşdırılmaların zəruriliyi xroniki xəstəliklərin effektiv müalicə üsullarının işlənib hazırlanması məqsədləri üçün mühüm əhəmiyyətə malikdir.

Açar sözlər: *Ağır metallar, bioakkumulyasiya, toksiklik, biokimyəvi pozuntular, kompleksyaratma qabiliyyəti, oksidləşdirici stres*

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Тяжелые металлы и их влияние на биохимические процессы в организме человека

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Экология и здоровье человека тесно взаимосвязаны. Тяжелые металлы являются приоритетными загрязнителями, поскольку они очень токсичны для живых организмов в относительно низких концентрациях. Тяжелые металлы рассматриваются Всемирной Организацией Здравоохранения как наиболее опасные для здоровья человека ксенобиотики. Данная статья посвящена обзору исследований по токсическому влиянию тяжелых металлов на биохимические процессы в организме и клиническому анализу вызванных ими болезней. Представлены различные механизмы токсичности тяжелых металлов на молекулярном уровне и, на достаточно большом материале, показана роль окислительного стресса в патогенезе заболеваний сердечно-сосудистой, нервной и дыхательной систем, связанных с действием тяжелых металлов. Анализ литературных данных свидетельствует о значительной роли и необходимости дальнейшего изучения биохимических механизмов развития патологических процессов при хроническом воздействии различных токсичных тяжелых металлов с целью разработки эффективных методов лечения хронических заболеваний.

Ключевые слова: *Тяжелые металлы, биоаккумуляция, токсичность, биохимические нарушения, комплексообразующая способность, окислительный стресс*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 47-52 (2020)

Effects of saffron (*Crocus sativus* L., *Iridaceae*) on the testosterone level in the blood and sexual behaviour of male rats

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Accepted for publication: 10 April 2020

The aim of the present research was to study the effects of Azerbaijan-grown saffron *Crocus sativus* L. *Iridaceae* on the testosterone level in the blood and sexual behaviour of normal male rats. The data obtained showed that saffron increased the blood testosterone level as compared to untreated controls, which did not receive saffron. *Per os* administration of saffron also led to marked stimulation of the proseptive and receptive components of the sexual behavior in male rats.

Keywords: *Saffron extract, sex hormones, testosterone, sexual behaviour, male rats, phyto-geroprotector*

INTRODUCTION

The objective of modern physiology, gerontology and medicine is to extend the active period of life and maintain reproductive health. Reproductive system is one of the most important systems of entire body, and age-related decline in reproductive functions is one of the manifestations of biological ageing in humans and animals (Anisimov, 2008; Colman et al., 2009; Svendsen et al., 2008; Wu et al., 2010). The activity of the reproductive system is directly related to age, and the level of sex hormones reflects and determines the biological age of a person. As the body ages, the function of the sex glands gradually decreases until it fades completely. Reproductive aging in males is characterized by a diminution in sexual behavior beginning in middle age.

Prevention and correction of premature aging are the key issues facing anti-ageing medicine and preventive geriatrics. They make the studies of properties of plant-derived medicines extremely relevant; according to WHO the global market for these substrates is steadily growing, in Europe and Central Asia, in particular (WHO monographs, 2010).

Saffron is of particular interest in this context, as it is known for its healing properties since ancient times. Modern pharmacological research methods make it possible to study the molecular mechanisms of effects of saffron, widely applied in ancient phytotherapy. By now, sufficient data has been accumulated to indicate the stimulating effects of saffron extract and its elements on sexual behavior of experimental animals (Agmo, 1997; Heidary et al., 2008; Hosseinzadeh et al., 2008; Shamsa et al., 2009). For example, H.Hosseinzadeh et al. studied the effects of the aqueous extract of saffron and its main components, safranal and crocin, on the sexual behavior of male rats. It was shown that safranal did not affect the sexual behavior of male rats, while *Crocus sativus* stigma aqueous extract and another safrant element, crocin, enhanced male sexual activity (Hosseinzadeh et al., 2008). M.Modaresi et al. showed the effectiveness of the saffron extract at a dose of 100 mg / kg on the pituitary-testis axis in mice (Modaresi, 2008). J.Ai et al. in the study of the effects of the aqueous extract from the stigmas of saffron on serum level of follicle-stimulating hormone, luteinizing hormone, progesterone and estrogen, as well as folliculogenesis in 45 adult rats, revealed that administration of the aqueous extract from the stigmas of saffron at a dose of 80 mg/kg significantly raised the serum level of all studied hormones as well as a number of basic, secondary and tertiary follicles in treated rats (Ai et al., 2009). Clinical studies also show the effectiveness of saffron in the treatment of premenstrual syndrome (PMS) (Agha-Hosseini et al., 2008). For example, the double-blind and placebo-

doi.org/10.29228/jlsb.41

controlled trial on women aged 20-45 years, who have regular menstrual cycle and PMS symptoms, revealed that oral administration of the saffron capsules at a dose of 30 mg/day (15 mg twice a day, morning and evening) for the duration of two menstrual cycles reduced the severity of PMS symptoms(Agha-Hosseini et al., 2008). Our study showed that the *per os* administration of alcoholic extract of saffron was able to decrease the FSH levels in blood of the 12-month-old rats as compared to that in the control group, involving the animals of the same age which have not received the saffron extract, and was close to the FSH levels reported for the 6-month rats. There was also an increase in number and body weight of pups from rats receiving the saffron extract prior to pairing with the intact males (Gashimova et al., 2017).

The objective of the research was to study the effects of the *Crocus sativus* L. *Iridaceae* stigma extraction some parameters of sexual behavior and blood level of testosterone in male rats.

MATERIAL AND METHODS

In the present study the saffron grown in the Bilgah village of the Absheron peninsula was used. The saffron stigma extract was obtained by a percolation method. The ethanol extract was filtered, the residue was washed with 75% alcohol and filtered again, then distilled off alcohol. The obtained liquid extract was further vacuum dried to concentrate to a dry residue. The yield of the active extract as viscous gum like substance constituted 56% of total mass of row material.

The tests was conducted on 45 Wistar rats, kept in standard cages (10 animals per cage) at a room temperature of 22±2° C. All animals were fed ad libitum with standard laboratory chow, and had an access to tap water.

The work was carried out in accordance with the international principles of the Helsinki Declaration on Humane Treatment of Animals, the Principles of Humanity set out in the European Community Directive (86/609/U), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Directive 2010/63/EU, 2012).

Male rats were divided into 3 groups: the 1st group included intact animals, the 2nd group (control) included animals that received physiological saline, and the 3rd group (experimental) included animals that received the saffron extract at the dose of 120 mg / kg for 21 days. The saffron extract and the physiological saline were administered to animals *per os* using a thin metal probe.

Sexual behavior in male rats was studied via standard 15-minute paired test (Buresh et al., 1991; Agmo, 1997) on the second day following the last administration of the saffron extract. The male rats were placed in the test chamber (measuring 50×35×25 cm) for 5 minutes prior to an introduction to a sexually receptive female rat. The experiments were performed under dim red light. Before being tested, the animals were kept in the dark for 4-5 hours. Each male was placed with 2 females. The females were returned to their cages after 10-minute exposure. The components of sexual activity were recorded visually for 15 minutes. We registered both the proceptive phase of sexual behavior (the latent period of the onset of sexual activity (LPS), the duration of sexual activity and the number of "emotional" approaches to the female) and the receptive sexual behavior (the number of female coverage's with/without intramission - the number of mounts (NM).

Upon completion of the experiments with saffron extract administration, the blood samples were collected from animals of the control and experimental groups. The blood testosterone level was studied on the 7th, 14th and 21st days of administration of the saffron extract. For the indicated time intervals, the blood sampling was carried out in the male rats of the control and experimental groups. Procedure was conducted in the morning (9-10 a.m.), blood was obtained from the tail vein under light (1 min) diethyl ether anesthesia. The level of testosterone was determined in the blood serum using hormonal test kits for enzyme immunoassay *in vitro* for mammals ("Pishtaz", Iran).

Data analysis was performed using Microsoft Excel statistical package. Statistical significance of differences was proven by Student's t-test.

RESULTS AND DISCUSSION

Analysis of the results of the study of saffron extract effect on sexual behavior of male rats re-

vealed positive dynamics in the proceptive and receptive sexual behavior. It was manifested by decrease of LPS and increase in the number of "emotional" approaches of the male towards the female and NM. The latent period before the initiation of the elements of courtship (licking, sniffing, grooming) in the experimental group dropped down compared to the controls (the experiment time lapse was 95.0±4.0 sec.; the control time lapse was 100.0±0.13 sec., $p<0.05$). Likewise, NM in saffron-treated male rats averaged 14.1±1.1, or 1.2 times higher than in the controls (Table 1).

The sexual behavior assessment shows that in male rats treated with saffron extract for 21 days, the proceptor behavior was activated at 15%, $p<0.05$. The indicators of receptive sexual behavior in experimental animals displayed a tendency towards growth compared to controls ($p<0.05$).

Hence, the results of the tests allow to conclude that saffron extract had the stimulatory effects on the sexual behavior of animals.

The male rats treated with saffron extract at a dose of 120mg/kg displayed a significant decrease in the temporal parameters of sexual behavior and an increase in the qualitative parameters of sexual motivation (Table 1). Consequently, it appeared rational to investigate the effects of saffron on male sex hormones content. For this purpose, blood samples were collected to register testosterone level in male rats on days 7, 14 and 21 of saffron extract administration.

Table 1. The effects of the saffron extract on the sexual behavior in male rats (M±m, n=10)

Groups of animals	Time, days	Sexual behavior		
		Duration of sexual activity, sec.	Numberof "emotional" approaches	Number of mounts (NM)
Intact		83.0±3.6	15.3±0.26	10.4±1.0
Control	7	75.0±3.9	14.1±0.14	10.2±0.9
	14	78.0±6.4	13.0±0.35	6.6±0.28
	21	95.0±4.0	16.2±0.24	11.2±1.0
Experiment	7	83.0±3.4	20.6±1.23	10.2± 0.9
	14	88.0±4.5	21.5±1.13	13.2±2.1
	21	100.0±3.1	22.2±0.17	14.1±1.1

The results revealed, that the saffron extract application for 21 days manifested a statistically significant increase of the total testosterone level in experimental animals at the end of the treatment period. The initial testosterone content in control animals was 1.35±0.44İU/L. After a 7-day administration of the saffron extract, it reached 1.38± 0.22 IU/L ($p<0.05$) (Table 2).

Consecutive changes in the total testosterone content occurred as follows: on day 14 of saffron extract treatment, the testosterone concentration was 1.42±0.14 IU/L ($p<0.05$); on day 21 of saffron extract administration it reached the point of 2.87±0.28 İU/L ($p<0.01$). It is noteworthy that the difference in testosterone levels on day 7 and day 21 of the treatment are statistically significant ($p<0.001$) (Table 2).

Table 2. The serum testosterone level in male rats under saffron extract treatment

Groups	Testosterone, İU/L		
Intact	1.35± 0.44		
	7th day	14th day	21st day
Controlgroup (saline)	1.35±0.44	1.38±0.22	1.4±0.24
Experimental group (the saffron extract)	1.38±0.22 p<0,05	1.42±0.14 p<0,05	2.27±0.28 p<0,001

Having evaluated the results, we conclude that the administration of saffron extract to male rats raised their sexual motivation and had positive influence on the serum testosterone level. Therefore, saffron extract application adds to maintaining of the reproductive functions of the body, which is biologically significant.

CONCLUSION

Saffron's unique medicinal properties largely owe to the diversity of ofbiologically active substances it contains (Kasumov et al., 2002; Abdullayev, 1993). The effects of saffron treatment observed in the course of this study could be explained by the direct impact of saffron and its components on the sex glands and the central nervous system.

There exists substantial literary evidence that the saffron extract is used to treat moderate depression (Akhondzadeh et al., 2004). Similar to the action of antidepressant drugs, safronal, one of saffron's major elements, inhibits serotonin re-up-

take and acts as a mild psychoactive drug (Georgiadou et al., 2012), thus contributing to the normalization of hypothalamic-pituitary-ovary system functions.

Our study showed that oral administration of the saffron extract at a dose of 50 mg/kg stabilized certain parameters of lipid metabolism, specifically, total lipids (TL), triglycerides (TG) and total cholesterol (TC). Henceforth, the administration of the saffron extract to animals receiving high-calorie diet promoted their weight loss, and reduced their blood levels of TL, TG and TC, compared to similar indicators in untreated animals (Gashimova et al., 2016), ultimately leading to lipid metabolism normalization.

In one of the tests (Verma et al., 1998), it was noted that a dose of 50 mg of saffron stigmas, dissolved in milk, administered twice a day, reduced the susceptibility of lipoproteins to oxidation, both in healthy control subjects and in patients with coronary heart disease. It is widely recognized, that lipoproteins are the transport forms of cholesterol. As cholesterol participates in the synthesis of vital hormones and all steroids, including testosterone and estradiol, as well as in the formation of cell membranes structure, its delivery to the body's peripheral tissues plays a key role (Colman et al., 2009). Saffron's ability to regulate lipid metabolism and reduce lipoproteins oxidizability may be one of the mechanisms behind the biological effects of saffron.

Taking into consideration that the imbalance of the neuroimmunoendocrine system lies at the base of premature aging, the effects of saffron on neuroendocrine relationships are of principal importance for the theory as much as the practice of the anti-aging medicine. Overall, the new findings of diverse pharmacological effects of the saffron extract open new horizons for the development of scientifically corroborated recommendations for application in practical medicine as a potential phyto-geroprotector.

The above-mentioned discoveries speak in favour of investigating the effects of saffron on the functioning of reproductive systems in both sexes, in experimental model, so as to understand the mechanisms that underlie pharmacological effects of saffron, and develop scientifically based recommendations for its application in anti-aging medicine and preventive geriatrics. Such an approach would make the study particularly pertinent, as it is focused on the experimental research of the effects of saffron on the activity of the reproductive system.

REFERENCES

Анисимов В.Н. (2008) Молекулярные и физиологические механизмы старения. СПб.: Наука, 481 с.

Буреш Я., Бурешова О., Хьюстон Д.П. (1991) Методики и основные эксперименты по изучению мозга и поведения. М.: 398с.

Гашимова У.Ф., Бабаев Х.Ф., Садых-заде Р.А. Шукюрова П.А. (2016) Потенциальные геропротективные свойства шафрана (*Crocus sativus, L.*). Врач, **6:** 35-38.

Гашимова У.Ф., Камилова Н.К., Бабаев Х.Ф., Шукюрова П.А., Гасанова С.И., Аббасов Р.Ю. (2017) Влияние шафрана (*Crocus sativus* L. *Iridaceae*) на уровень фолликулостимулирующего гормона в крови у самок крыс, количество потомства и динамику массы тела у крысят. *Успехи геронтологии*, **30(3):** 436-441.

Директива 2010/63/ EU европейского парламента и совета Европейского союза по охране животных, используемых в научных целях (2012). СПб.: 48 с.

Касумов Ф.Ю., Несруллаева Г.М., Абдуллаева И.М. (2002) Биологическая характеристика и химический состав шафрана посевного. *Актуальные проблемы офтальмологии. Баку,* с. 95-97.

Кольман Я., Рём К.-Г. (2009) Наглядная биохимия (пер. с нем.). М.: Мир.

Монографии ВОЗ о лекарственных растениях, широко используемых в Новых Независимых Государствах (ННГ) (2010) Изд. Всемирная организация здравоохранения: 464 с.

Abdullayev F.I. (1993) Biological effects of saffron. *BioFactors*, **4:** 83-86.

Agmo A. (1997) Male rat sexual behavior. *Brain Res. Brain Res. Protoc.*, **1 (2):** 203–209.

Agha-Hosseini M., Kashani L., Aleyaseen A.et al. (2008) Crocus sativus L. (saffron) in the treatment of premenstrual syndrome: a double-blind, randomized and placebo-controlled trial. *BJOG,* **115 (4):** 515-519.

Akhondzadeh S., Fallah-Pour H., Afkham K.et al. (2004) Comparison of *Crocus sativus* L. and imipramine in the treatment on mild of moderate depression: a pilot double-blind randomized trial [ISRCT N 45683816]. *BMC Complement Altern. Med*., **2:** 6771.

Ai J., Nekooeian A.A., Takhshid M.A., Mostafizi N. et al. (2009) Effect of aqueous extract of *Crocus sativus* L. (Saffron) stigma on serum levels of gonadotropins and folliculo-genesis in adult rats. *Journal of Applied Animal Research*, **35:** 49-52.

Georgiadou G, Tarantilis PA, Pitsikas N. (2012) Effects of the active constituents of Crocus Sativus L., crocins, in an animal model of obsessive-compulsive disorder. *Neurosci. Lett*., **528 (1):**27-30.

Heidary M., Vahhabi S., Reza Nejadi J. et al. (2008) Effect of saffron on semen parameters of infertile men. *Urol*. J., **4 (5):** 255-259.

Hosseinzadeh H., Zeall T., Sadeghi A. (2008) The effect of saffron, *Crocus sativus* stigma, extract and its constituents safranal and crocin on sexual behaviors in normal male rats. *Phytomedicine***, 15 (6-7):** 491-495.

Modaresi M., Messripour M., Asadi Marghmaleki M. et al. (2008) Effect of saffron (*Crocus sativus*) extract on level of FSH, LH and testosterone in mice. *ZUMS Journal*, **16 (63):** 11-18.

Shamsa A., Hosseinzadeh H., Molaei M. et al. (2009) Evaluation of *Crocus sativus* L. (saffron) on male erectile dysfunction: a pilot study. *Phytomedicine***, 8 (16):** 690-693.

Svendsen K., Schultz A. (2008) Seksuell dysfunksjon hos men. *Tidsskrift Norsk Legeforening*, **128:** 448-452.

Verma S.K., Bordia A. (1998) Antioxidant property of Saffron in man. *Indian J. Med. Sci*., **52 (5):** 205-207.

Wu D., Gore A.C. (2010) Changes in androgen receptor, estrogen receptor alpha, and sexual behavior with aging and testosterone in male rats. *Norm. Behav*., **58 (2):** 306-316.

Erkək siçovulların qanında testosteronun səviyyəsinə və cinsi davranışa zəfəranın (*Crocus satıvus* L. *Irıdaceae*) təsırı

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Tədqiqat işinin əsas məqsədi Abşeronun Bilgəh kəndində becərilən endemic zəfəranın (*Crocus sativus L. İridaceae*) dişicik ağızcığından alınmış ekstraktının normal erkək siçovulların qanında testosteronun səviyyəsinə və çütləşmə davranışlarının bəzi göstəricilərinə təsirinin öyrənilməsidir. Aparılmış tədqiqatlardan məlum olur ki, erkək siçovullara zəfəran ekstraktının verilməsi heyvanalrın qanında testosteronun səviyyəsini nəzarət qrupu ilə müqayisədə artırdığını göstərdi. Alınmış nəticələrə əsaslanaraq həmçinin demək olar ki, zəfəran ekstraktı cinsi davranışlara stimullaşdırıcı təsir göstərir.

Açar sözlər: *Zəfəran ekstraktı, cinsi hormonlar, testosteron, cinsi davranış, erkək siçovullar, fitogeroprotektor*

Влияние шафрана (*Crocus sativus* L. *Iridaceae*) на уровень тестостерона в крови и половое поведение у крыс-самцов

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Целью настоящего исследования было изучение влияния экстракта, полученного из рылец шафрана, произрастающего в Азербайджане, на уровень тестостерона в крови и некоторые параметры полового поведения нормальных самцов крыс. Полученные данные показали, что шафран повышает уровень тестостерона в крови по сравнению с контрольной группой, не получавших экстракт. Введение шафрана также приводило к выраженной стимуляции процептивных и рецептивных компонентов полового поведения у самцов крыс.

Ключевые слова: *Экстракт шафрана, половые гормоны, тестостерон, половое поведение, самцы крыс, фитогеропротектор*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 53-58 (2020)

Study of the activity of serotonin-modulating anticonsolidation protein in regulation of proliferation and differentiation of embryonic cells of *Xenopus laevis*

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Accepted for publication: 15 May 2020

The article concerns the studies of the activity of serotonin-modulating anticonsolidation protein (SMAP), being in linear relations with serotonin, in regulation of cell proliferation and differentiation on the embryonic model of *Xenopus laevis*. In the 1st series of studies the dynamics of SMAP level in the embryos of *Xenopus laevis* throughout the all stages of embryogenesis and metamorphosis was pursued with application of indirect ELISA-test. Beginning from the blastula stage till the end of the neurula its level remained unchanged. On the following stages continuous downregulation of the SMAP level, interrupted as a slight upregulation between the 37th stage and onset of the 39th stage, was observed. In the 2nd series of studies, incubation of the embryos of *Xenopus laevis* on the blastula and gastrula stages in fresh water containing SMAP at a dose of 50 and 100 μg/ml led to delay in development and, finally, to death of all the embryos within 4 days of the observation. In the 3rd series of the studies, blocking of SMAP activity with the anti-SMAP polyclonal antibodies at a dose of 50 μg/ml in the embryos of *Xenopus laevis*, being on the 37th stage of development, resulted in their accelerated passing (by two stages earlier) of the metamorphosis stage relative to the intact and control groups. Hence, if on the initial stages of embryogenesis SMAP demonstrates its cytostatic activity, on the metamorphosis stage antibodies-mediated blockade of its activity, conversely, leads to accelerated passing of embryonic cells through cell differentiation.

Keywords: *Serotonin-modulating anticonsolidation protein, antibodies, embryogenesis, metamorphosis, Xenopus laevis*

INTRODUCTION

Certain stages of embryonic processes have high level of resemblance with carcinogenesis processes, particularly in high proliferative activity of their composing cellular elements. Along with it, the advantage of studying embryonic stages relatively to cancer processes concludes in that that processes of cell proliferation and differentiation are diverged over time, mostly within different embryonic stages. So, utilization of embryonic models makes possible to elaborating the approaches directed purposefully and separately on the processes of cell proliferation and differentiation. Issuing from high proliferative potential of the cancer cells, it is clear why suppressive effects on cell proliferation and the remedies possessing with such activities are under meticulous attention of the most researches dealing with the studies of carcinogenesis and seeking ways of combating with tumor. Along with it, from the first glance, the unusual interest to the processes of cell differentiation and study of the underlying molecular mechanisms, coming from the cancer-absorbed researchers, is related to a problem of the cancer stem cells constituting serious obstacle in treatment of malignant tumors (Fulawka1 et al., 2014; Cojoc et al., 2015; Pan et al., 2015). The problem of their eradication is complicated by the fact that broadly applied chemotherapeutic preparations are ineffective against these cells.

Sometimes the struggle with cancer stem cells is likened to the struggle with weeds: you can chop off the weeds' stalks many times, but

doi.org/10.29228/jlsb.42

they will grow again and again, so far their roots still remain in the soil. The same is referred to the cancer stem cells in the sense that the role of woods' stalk in this case belongs to mature malignant tumor cells, while the role of woods' roots – to the cancer stem cells themselves.

The goal of the present study was application of different stages of embryonic and early stages of development of *Xenopus laevis* for the purpose of elaborating preparations directed either to suppression of cell proliferation, or, in opposite, to promoting forced cell differentiation. This goal was achieved through application of serotonin-modulating anticonsolidation protein (SMAP) and anti-SMAP polyclonal antibodies for the purpose of blocking its activity in different stages of the embryonic and early stages of development of *Xenopus laevis.*

MATERIALS AND METHODS

Biochemical techniques. SMAP, being in linear relations with serotonin, was purified from the cow brains with application of two-step purification procedure as had been described earlier (Mekhtiev, 2000): 1) partial precipitation with sodium sulfate in the range of 0-40% concentration; 2) gel-chromatography on the column (3.0 X 60.0 cm) of Sephadex G-150. SMAP purification was carried out under the screening control of the indirect ELISA-test (Catty, 1991) with application of anti-SMAP rabbit immuneglobulins to selecting the SMAP-enriched protein fractions. The protein purity was checked by electrophoresis in polyacrylamide gel.

The anti-SMAP polyclonal immunoglobulins were produced through immunization of the rabbits with SMAP, using 300 μg of the protein always in mixture with the complete Freund adjuvant. SMAP and anti-SMAP immunoglobulins were frozen and kept under -70°C.

The content of SMAP was determined in the embryos and tadpoles of *Xenopus laevis* by the indirect ELISA-test realized on the polystyrene plates of moderate adsorption (Catty, 1991). The samples were homogenized and water-soluble proteins were extracted and used as antigens in the ELISA-test. Specific polyclonal rabbit anti-SMAP antibodies were used as the first antibodies, while the anti-rabbit goat immunoglobulins, coupled with covalent bonds with horseradish peroxidase, were used as the second antibodies. Orthophenylendiamine was used as a substrate for peroxidase to visualize the results of the reaction. The reaction was stopped by adding 3 M NaOH and the results were transformed into digital form by the ELISA-test reader of the model of "Molecular Devices Spectra Max 250" (MTX Lab Systems, Inc., USA) at the wavelength of 492 nm (wavelength of reference 630 nm) and analyzed with application of the t-Student criterion.

Embryonic technique. The embryonic studies were conducted on *Xenopus laevis.* The eggs from the sexually mature animals were obtained after injecting them with human gonadotropin at a dose of 150 units for males and 350 units for females.

In the 1st series of studies after several hours since gonadotropin administration, females released roe which was fertilized by males. Simultaneously after roe fertilization samples of the embryos were taken from different stages of embryonic development (stages 1-2, 7, 9, 11, 13, 20, 22, 28), pre-metamorphosis, metamorphosis and post-metamorphosis (stages 37, 42, 44, 45, 49, 53) for evaluation of the level of SMAP with application of the indirect ELISA-test and anti-SMAP polyclonal immunoglobulins. For evaluation of SMAP 10 samples of specimens within each stage were taken. The results were averaged within each group and differences between adjacent stages were evaluated on t-Student's criterion.

In the 2nd series of studies the embryos, being on the blastula and gastrula stages, were placed into the Petri dish with fresh water containing different preparations. The embryos were culled into three groups: 1) intact group (n=17), 2) control group – SMAP and anti-SMAP antibodies – both at a concentration 50 μg/ml (n=15), and 3) experimental group – SMAP at a concentration of 50 and 100 μg/ml (n=17).

In the 3rd series of studies the animals, being on the 37th stage of development (beginning from the late tailbud stage), were placed into the Petri dish with fresh water containing different preparations. The embryos were culled into four groups: 1) intact group (n=13), 2) control group – SMAP and anti-SMAP antibodies - both at a concentration of 50 μg/ml (n=13), 3) 1st experimental group –

SMAP at a concentration of 50 μg/ml (n=13), 4) 2nd experimental group – SMAP at a concentration of 100 μg/ml (n=13), and 5) 3rd experimental group – anti-SMAP antibodies – at a concentration of 50 μg/ml (n=13). After 24 h the animals were transferred into the tanks with fresh water with regulated temperature and air supply. On the basis of elaborated tables of early stages of development of *Xenopus laevis* (Sive et al., 2010), registration of passing metamorphosis stage by the animals of different groups was carried out. Particularly, definition of passing of this stage by the animals was realized on the basis of degree of the tail resorption, advent of the precursors (buds) of the hind legs, changes in the shape of mouth and other second-order morphological changes typical to mature animals.

The results were averaged within each group and differences between different groups were evaluated on Wilkoxon-Mann-Whitney's U-criterion.

RESULTS

Dynamics of SMAP levels on embryonic stages and metamorphosis in Xenopus laevis

In the 1st series of studies the results revealed the dynamics of the level of SMAP in the organism of the animals throughout the early stages of ontogenesis (embryogenesis and metamorphosis) of *Xenopus laevis*. It was noticed that after slight downregulation of SMAP immediately after fertilization of the roe, on the forthcoming stages till finalization of the neurula its level remained unchanged, on the same values. Thereafter continuous and significant ($p<0.01$ and $p<0.001$) downregulation of the SMAP level, interrupted once in the form of its slight upregulation in the interval between middle of the late tailbud stage (37th stage) and onset of the pre-metamorphosis stage (39th stage; Fig.), was observed.

Role of SMAP in regulation of embryonic development and metamorphosis in Xenopus laevis. In the 2nd series of studies incubation of the embryos of *Xenopus laevis* on the blastula and gastrula stages in fresh water containing SMAP at a dose of 50 and 100 μg/ml led to delay of their development and, finally, to death of all the embryos of this group within 4 days of observation. At the same time the embryos of the intact and control groups left alive and developed normally, without lethality of any specimens.

In the 3rd series of studies blocking of SMAP activity with the anti-SMAP polyclonal antibodies in the 3rd experimental group realized significant effect on the rate of animal passing through embryogenesis.

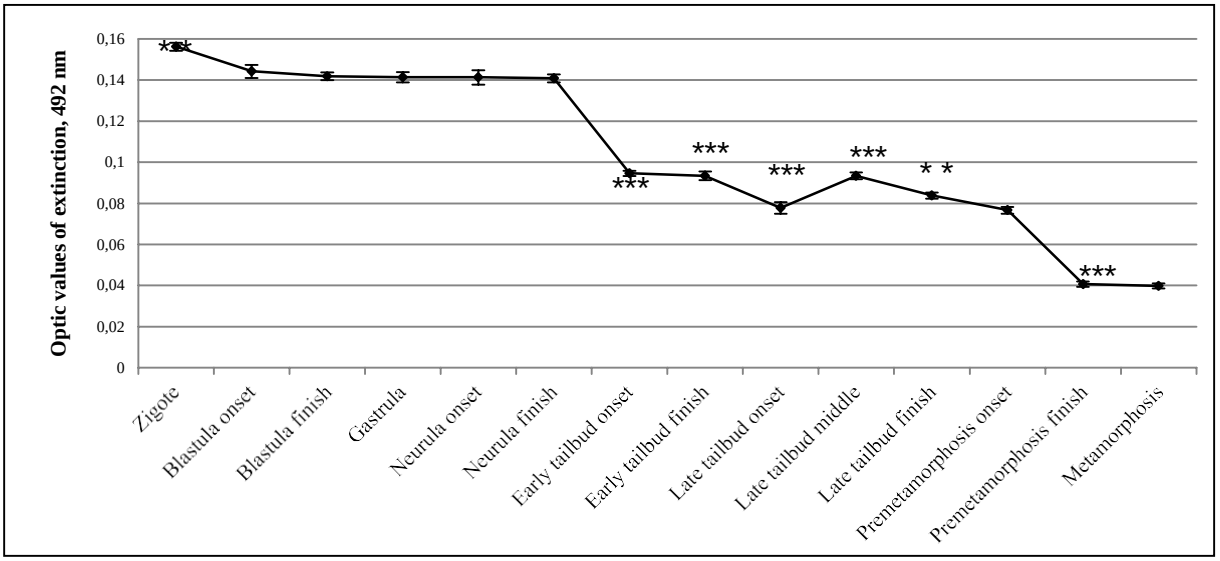

Figure. Changes of SMAP level in the early stages of ontogenesis in *Xenopus laevis*. **- $p<0.01$; *** - $p<0.001$ relatively to the preceding point.

Particularly, it was shown that single addition of the anti-SMAP antibodies at a dose of 50 μg/ml into the incubation milieu of the embryos of *Xenopus laevis*, being on the 37th stage of development, after 14 days resulted in their passing ahead (two stages earlier) of the metamorphosis stage (50 stage) relatively to the rate of passing of this stage by the animals of the intact (48th stage) and control (48th stage) groups (p=0.01). Along with it, animals of the 1st and 2nd experimental groups under effects of SMAP of both doses were on the 49th stage of development passing ahead metamorphosis by one stage earlier relatively to the animals of intact and control groups (p=0.01).

Hence, up to the end of the neurula stage of embryogenesis of *Xenopus laevis* the level of SMAP in the organism remains unchanged, while its continuous downregulation on the next stages is observed. Addition of SMAP to the embryos at the stages of blastula and gastrula leads to delay of embryonic development and, finally, to death of all the embryos. Blocking of SMAP activity by anti-SMAP antibodies on the stage of pre-metamorphosis of *Xenopus laevis* leads to noticeable passing ahead of the metamorphosis stage by the embryos.

DISCUSSION

The results of the studies indicate to close involvement of SMAP, realizing serotonin functions on sub-cellular level, in the processes of cell prolipheration and differentiation. As the initial stages of embryogenesis are characterized with high indexes of cell prolipheration, remaining of the SMAP level stable, in the form of smooth horizontal line during theses stages up to the end of neurula stage shows that such unchanged amount of SMAP is required for molecular support of intensive cell proliferation.

As it is known from the literature, on the next after neurula stages of embryogenesis and initial stages of development including metamorphosis, decline of cell proliferation and, in opposite, step-by-step strengthening of cell differentiation are observed (Sive et al., 2010). Gradual downregulation of SMAP on the said stages indicates that its level is synchronized negatively to realization and/or regulation of differentiation processes, thus giving grounds to making a conclusion that SMAP itself is engaged in negative regulation of cell differentiation, probably, through switching on differentiation-launching genes on the background of SMAP downregulation.

The above stated conclusions, issuing from the results of the 1st series of the studies, were confirmed later by the results of the undertaken 2nd and 3rd series. Particularly, artificial upregulation of the SMAP level in the embryos through its addition to the incubation milieu of embryos, staying on the blastula and gastrula stages, under the both applied doses brought to cessation of the embryo development and total death of the embryos. Hece, SMAP upregulation on the initial stages of embryogenesis realizes cytotoxic effects on the embryonic cells.

The observed cytotoxic effects of SMAP on the onset of embryogenesis of *Xenopus laevis* may be related to induction by SMAP of conformational changes of chromatin transducting it into the condensed inactive form. This idea is confirmed by our ealier studies in which SMAP administration to the sturgeon juveniles led to significant downregulation (by over 50%) of the level of mutagenic changes in the somatic cells induced by soil sediments from Baku Bay containing high levels of heavy metals and polyaromatic hydrocarbons relatively to the control group kept under the similar polluted conditions. In the control animals this contamination induced 5-times elevation of mutations relatively to the intact animals kept in fresh water (Mekhtiev, Movsum-zadeh, 2008). These earlier obtained data give grounds to make a conclusion that SMAP brings chromatin to the condensed, folded state, this way providing its protection from the effects of adverse factors.

Along with it, antibodies-mediated blockade of SMAP activity on the 37th stage of embryonic development significantly accelerated passing through metamorphosis (by two stages earlier) the embryos of the 3rd experimental group relatively to the intact and control animals, this way supporting the idea of existing significant negative regulation of the differentiation processes of embryonic cells by SMAP, coming from its downregulation on post-neurula stages of development. Besides, such accelerated passing of metamorphosis, though in somehow fainter degree (by one stage earlier), was noticed for the effects of SMAP at both studied doses.

However, these effects of SMAP itself are, probably, due to the phenomenon of down-regulation of SMAP-accepting receptors resulting from their internalization into the embryonic cells under the effect of applied SMAP. Hence, these results give grounds in the future for application of anti-SMAP antibodies for the purpose of forced differentiation of immature embryonic-resembling cells, particularly cancer stem cells, having many common features with the embryonic cells. The idea of possible impact on cancer stem cells through modulating microenvironmental stimuli, leading to their differentiation, has been put forward by other researchers (Carry, 1991; Sell, 2004; Radosevich et al., 2015).

REFERENCES

Catty D. (1991) Antibodies. A practical approach. Oxford, New York, Tokyo: Oxford University Press, **Vol. 2:** 384 p.

Cojoc M., Mäbert K., Muders M.H., Dubrovska A. (2015) A role for cancer stem cells in therapy resistance: Cellular and molecular mechanisms. *Seminars in Cancer Biology*, **31:**16-27.

Fulawka1 L., Donizy P., Halon A. (2014) Cancer stem cells – the current status of an old concept: literature review and clinical approaches. *Biological Research*, **47(1):** 66-75.

Mekhtiev A.A. (2000) Revealing in the rat brain protein, possessing with anticonsolidation properties. *Bulletin of Exper. Biol. Med*., **129(8):** 147-150.

Mekhtiev A.A., Movsum-zadeh S.K. (2008) Antimutagenic activity of serotonergic system and underlying mechanisms in sturgeon juveniles (*Acipenser gueldenstaedti persicus*) and goldfish (*Carassius auratus*). *J. Evol. Biochem. Physiol*., **44(5):** 476-481.

Pan Q., Li Q., Liu Sh., Ning N., Zhang X., Xu Y, Chang A.E., Wicha M.S. (2015) Concise Review: Targeting cancer stem cells using immunologic approaches. *Stem Cells*, **33:** 2085-2092.

Radosevich J., Aqil M., Bassiony M. (2015) Rethinking the tumor stem cell theory. *Tumor Biology*, **36(Suppl. 1):** S24.

Sell S. (2004) Stem cell origin of cancer and differentiation therapy. *Critical Reviews in Oncology/Hematology*, **51:** 1-28.

Sive H.L., Grainger R.M., Harland R.M. (2010) Early development of *Xenopus laevis.* A laboratory manual. New York: Cold Spring Harbor Laboratory Press, 340 p.

Mahmızlı qurbağaların (X*enopus laevıs*) embrional hüceyrələrində proliferasiya və differensasiyanın tənzimlənməsində serotonin modulyasiyalı antikonsolidasiya zülalın rolunun öyrənilməsi

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Məqalə mahmızlı qurbağaların embrional modelində hüceyrələrin proliferasiya və differensasiya proseslərinin tənzimlənilmsində serotoninin səviyyəsindən düz mütanasib əlaqədə olan serotonin- modullu antikonsolidasiya zülalın (SMAZ) töhfəsinin öyrənilməsinə həsr edilib. Tədqiqatların 1-ci seriyasında mahmızlı qurbağaların embrionlarında bütün embriogenez və metamorfoz mərhələləri boyunca birbaşa olmayan immuno-enzim analiz metodu vasitəsilə SMAZ-ın dəyişmə səviyyəsinin xarakteri izlənilmişdir. Blastula mərhələsindən başlayaraq neyrula mərhələsinin sonunadək SMAZ-ın səviyyəsi dəyişməz qalmaqdadır. Sonrakı mərhələlərdən SMAZ-ın səviyyəsi (37-ci və 39-cu mərhələlərdə azacıq artmaqla) fasiləsiz azalması müşahidə olunur. Tədqiqatların 2-ci seriyasında SMAZ-ın 50 və 100 mkq/ml dozalı miqdarı olan adi suda mahmızlı qurbağaların blastula və qastrula mərhələlərində olan embrionları inkubasiya edilmiş və nəticədə 4 günlük müşahidələr zamanı bütün embrionların tələf olması qeydə alınmışdır. Tədqiqat-

ların 3-cü seriyasında 50 mkq/ml dozada SMAZ-a qarşı poliklonal anticismlərin köməkliyi ilə SMAZ-ın aktivliyinin blokadası edildi. Təcrübə qrupunu intakt və kontrol qruplarla müqayisədə 37-ci mərhələdən başlayaraq metamorfoz mərhələlərin (2 mərhələ qabaqlamaqla) sürətli keçməsinə gətirmişdir. Beləliklə, SMAZ embriogenezin ilkin mərhələlərində sitostatik aktivlik göstərirsə, metamorfoz mərhələsində onun (SMAZ-ın) anticismlər vasitəsilə blokadası əksinə, hüceyrə differensasiyası mərhələli keçidi embrional hüceyrələrdə sürətləndirir.

Açar sözlər: *Serotonin-modullu antikonsolidasiya zülalı, anticisimlər, embriogenez, metamorfoz, mahmızlı qurbağalar*

Изучение роли серотонин-модулируемого антиконсолидационного белка в регуляции пролиферации и дифференциации эмбриональных клеток шпорцевых лягушек (*Xenopus laevis)*

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Статья посвящена изучению вклада серотонин-модулируемого антиконсолидационного белка (СМАБ), находящегося в прямой зависимости от уровня серотонина, в регуляцию процессов клеточной пролиферации и дифференциации в эмбриональной модели шпорцевых лягушек *Xenopus laevis.* В 1-й серии исследований с помощью метода непрямого иммуноферментного анализа был прослежен характер изменения уровня СМАБ в эмбрионах шпорцевых лягушек на протяжении всех стадий эмбриогенеза и метаморфоза. Начиная со стадии бластулы, вплоть до конца нейрулы, его уровень оставался неизменным. На последующих стадиях наблюдалось непрерывное снижение уровня СМАБ, прерванное в виде небольшого увеличения его между 37-й и началом 39-й стадии. Во 2-й серии исследований эмбрионы шпорцевых лягушек на стадиях бластулы и гаструлы инкубировались в пресной воде, содержавшей СМАБ в дозе 50 и 100 мкг/мл, что приводило к задержке в развитии и, в конечном итоге, к гибели всех эмбрионов в течение четырехдневного наблюдения. В 3-й серии, блокада активности СМАБ у эмбрионов шпорцевых лягушек с помощью поликлональных антител в дозе 50 мкг/мл, начиная с 37-й стадии развития, приводила к ускоренному прохождению стадии метаморфоза (на 2 стадии быстрее) по сравнению с интактной и контрольной группами. Таким образом, если в начальной стадии эмбриогенеза СМАБ демонстрирует цитостатическую активность, на стадии метаморфоза блокада его активности с помощью антител, напротив, приводит к ускоренному прохождению эмбриональных клеток через стадии клеточной дифференциации.

Ключевые слова: *Серотонин-модулируемый антиконсолидационный белок, антитела, эмбриогенез, метаморфоз, шпорцевые лягушки*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 59-65 (2020)

Dopplerographic and electrophysiologic studies of retinitis pigmentosa in young people

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Accepted for publication: 22 May 2020

The work concerns the study of retinitis pigmentosa (RP) – a hereditary generalized retinal dystrophy. Purpose: to study the changes in hemodynamic parameters in the eye vessels and ERG indexes and to implement a correlation analysis of young patients with various stages of RP. 100 patients (200 eyes) at the age from 15 to 24 years old with RP were examined, among them 41 patients – women, 59 patients – men. Patients were culled into 3 groups according to the functional disorders: I – 31 patients (62 eyes) with initial stage of RP, II – 40 patients (80 eyes) with medium severe stage of RP, III – 29 patients (58 eyes) with severe stage of RP. All patients underwent CDI on the apparatus Toshiba "NEMIO XG SSA-580A" (Japan) with ultrasound probe 8 mHz and ERG (standard combined, 30 Hz flicker, photopic) on the apparatus ROLAND CONSULT Super Color Ganz feld Q450 SC (Germany). The results of CDI and ERG indicate statistically significant changes in medium severe and severe stages of RP, also point to the disorders referred to the initial stage. CDI in the patients with RP allows detecting hemodynamic disorders timely. Analysis of ERG parameters indicates an impairment of photoreceptors function in parallel to ocular blood flow disorders. So, CDI and ERG are required for the purposes of early diagnostics and monitoring of patients with RP as well as for therapeutic and preventive measures.

Keywords: *Retinitis pigmentosa (RP), colour Doppler imaging (CDI), electroretinography (ERG)*

INTRODUCTION

In spite of rapid scientific and technical progress in all the branches of medicine, to date there are the fields in which undertaken attempts to suspend pathological process or restore impaired functions are not effective. One group of such serious pathologies in ophthalmology is hereditary diseases. Retinitis pigmentosa (RP) is a hereditary generalized retinal dystrophy. This pathology results in vision loss to the extent of blindness. The solution of this problem has not been found yet, hence, RP is the cause of disability of children and adults. (Гашимова и др., 2010; Шамшинова, 2001; Щуко и др., 2010; Hamblion et al., 2010).

There are many works aimed to the study of various aspects of RP (Robson et al., 2006; Sandberg et al., 2011; Eden et al., 2013; Riera et al., 2017). In recent years, the problem of vascular disorders in RP has also been studied. In ophthalmology and dopplerographic ultrasound examinations are widely used for evaluation of the vessel conditions. Colour Doppler imaging (CDI) is one of the methods of Doppler ultrasonography, which gives the opportunity to get reliable information about the eye hemodynamics. This method is non-invasive, convenient for implementation, does not require special training. However, to date, little studies are known concerning analysis the eye vessels hemodynamics in RP (Касимов и др., 2012; Parodi et al., 2017).

ERG is a leading method for the evaluation of functional condition of the retina, in particular photoreceptors, and gives an opportunity of detecting not only marked dystrophic changes of the retina, but also of diagnosing the functional disorders, which precede clinical manifestations of RP (Dolan et al., 2002; Seeliger et al., 1998; Marmor

doi.org/10.29228/jlsb.43

et al., 2009). For this reason, it is noteworthy implementation of complex of Dopplerographic and electrophysiologic studies in determining the severity of dystrophic process in RP (Киселева и др., 2015; Zhang et al., 2013).

The work objective concludes in study of the changes in hemodynamic parameters in the eye vessels and ERG indexes and in implementation of a correlation analysis in young patients with various stages of RP**.**

MATERIALS AND METHODS

100 patients (200 eyes) with RP at the ages of 15-24 were examined. Among them, 41 patients were female and 59 were male. The patients were culled into 3 groups. Group I included 31 patients (62 eyes) with initial stage of RP. During ophthalmoscopy, these patients had a deposition of single characteristic "bone bodies" on extreme and middle periphery of the eye fundus, the field of vision was restricted concentrically up to 40 degrees, and visual acuity in average referred to 0.95 ± 0.03. Group II included 40 patients (80 eyes) with medium severe stage of RP. During ophthalmoscopy, these patients had marked pigmentation by the type of "bone bodies" on extreme and middle periphery of the eye fundus, the field of vision was restricted concentrically from 40 to 20 degrees, and visual acuity at the average was 0.50±0.26. Group III included 29 patients (58 eyes) with severe RP stage. In this group of patients, during ophthalmoscopy, the marked pigmentation of the "bone bodies" type on extreme and middle periphery of the eye fundus was also revealed, the field of vision was restricted concentrically below 20 degrees, and visual acuity at the average was equal to 0.2±0.16.

Ophthalmological research methods included visometry, refractometry, tonometry, perimetry, biomicroscopy, and ophthalmoscopy. As well, all the patients underwent ERG – total, rhythmic 30 Hz (RERG), macular (MERG) – with application of the device ROLAND CONSULT Super Colour Ganz feld Q450 SC (Germany).

Ultrasound examinations included B-scanning of the eyeball and Doppler ultrasonography of the eye vessels using the CDI method on the apparatus Toshiba “NEMIO XG SSA-580A” (Japan) with ultrasound probe 8 mHz. Ophthalmic artery (OA), central retinal artery (CRA) and posterior short ciliary arteries (PSCA) were studied. The velocity parameters Vmax (maximum systolic velocity of blood flow), Vmin (final diastolic velocity of blood flow) and RI (resistance index) were determined in these vessels. In order to determine standard age indicators, Doppler ultrasound examination of 50 practically healthy volunteers of 15-24-year-old, included into the control group, was performed.

Statistical data processing was performed using Microsoft Excel-2010. The reliability of the results was evaluated using the Student's t-test, and the differences between average values were accepted as reliable at $p<0.05$. In order to analysing statistical interrelation between the recorded parameters, the Pearson correlation calculation method was used.

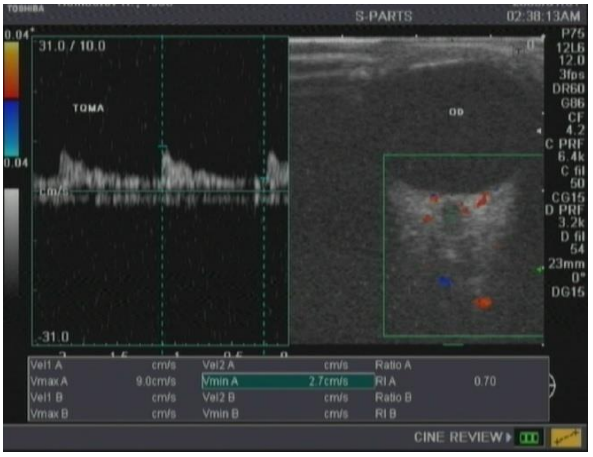
RESULTS AND DISCUSSION

In accordance to the results of CDI, various changes of hemodynamic parameters were observed in all the studied vessels. In OA, significant decrease in RI was found in the patients of group III. The values of Vmax and Vmin parameters of their vessels corresponded to the normative values in all studied groups. In CRA and PSCA significant changes in hemodynamic parameters were found in the patients of all the groups. Moreover, the most marked disturbances were observed in the patients of groups II and III (Table 1; Figure 1 and 2).

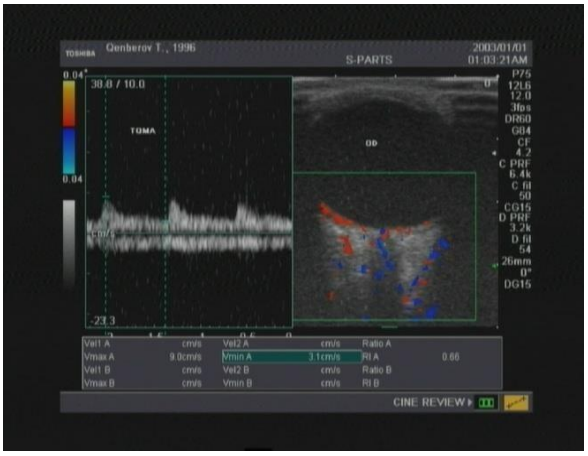
Thus, in OA of patients of group III, RI in average was equal to 0.71±0.02, and it was significant decrease with $p < 0.01$ (Table 1).

In CRA in the patients group II, Vmax was decreased significantly up to 8.1±0.07 cm/sec with $p<0.01$, and in group III – up to 7.0±0.1 with $p<0,001$. In the same vessels, Vmin in group II corresponded to average 3.05±0.1, and in group III – 2.2±0.01, both changes were significant with $p<0.01$. RI in the CRA was also recorded as reduced in all the groups and at average was 0.62±0.1 ($p<0.01$) in group II, and 0.60±0.03 ($p < 0.001$) in group III (Table 1).

(a) Group I patient



(b) Group II patient



(c) Group III patient

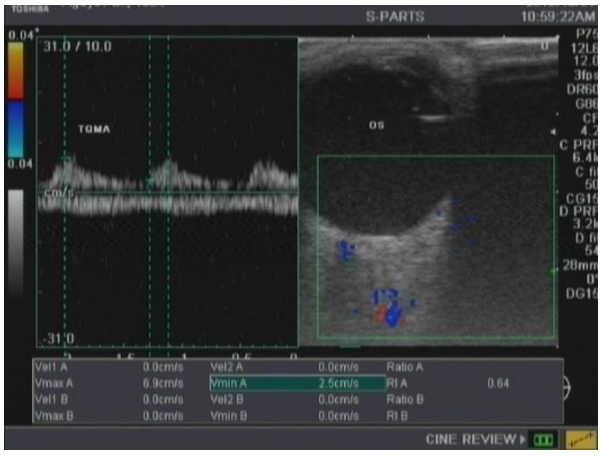
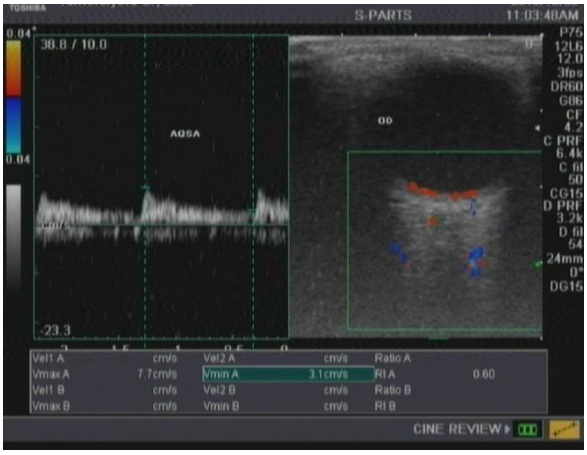
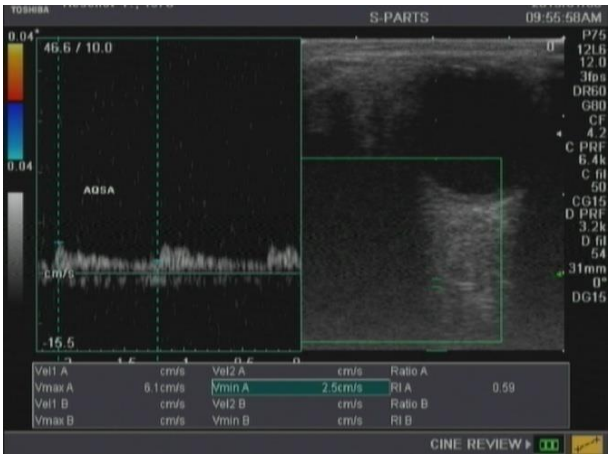


Figure 1. Sonograms of the CDI of the CRA.

(a) Group I patient



(b) Group II patient



(c) Group III patient

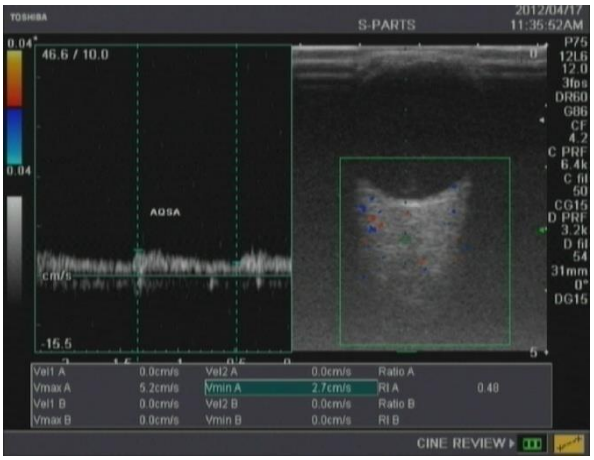


Figure 2. Sonograms of the CDI of the PSCA.

Table 1. Results of CDI in RP

Vessels	Parameters	I группа	II группа	III группа	Норма
OA	Vmax (sm/sec)	37.01 ± 0.7	37.25 ± 0.2	37.0±0.2	37.3 ±0.3
	Vmin (sm/sec)	8.9 ± 0.01	9.01 ± 0.1	9.1 ± 0.1	9.47 ±0.12
	RI	0.74±0.1	0.74±0.04	0.71±0.02**	0.75 ±0.002
CRA	Vmax (sm/sec)	11.2± 0.5*	8.1±0.07**	7.0±0.1***	13.2 ±0.1
	Vmin (sm/sec)	3.4 ± 0.02*	3.05 ± 0.1**	2.2 ± 0.01**	3.82 ±0.04
	RI	0.66±0.05**	0.62±0.1**	0.60±0.03***	0.71 ±0.002
PSCA	Vmax (sm/sec)	7.5 ± 0.09**	6.2±0.01***	5.0±0.8***	9.5 ±0.1
	Vmin (sm/sec)	3.2 ±0.2**	3.1± 0.05**	2.6±0.01***	3.67 ±0.04
	RI	0.55±0.01**	0.52±0.01***	0.47±0.02***	0.61 ±0.002

* - p < 0.05; ** - p < 0.01; ***- p < 0.001 – statistically significant difference in relation to the norm

Table 2. Indicators of total ERG, MERG and RERG

Indicators			Group I	Group II	Group III	Norm
Total ERG	a- wave	amplitude (μV)	98.3±11.4*	49.6±8.0***	15.3±7.1***	155-356
		latency (ms)	20.3±3.9	22.8± 4.2*	23.5±4.0*	14-22
	b-wave	amplitude (μV)	270.9±10.1*	114.4±8.2***	40.1±7.0***	290-654
		latency (ms)	43.5±3.1	44.7±2.3	51.0±2.2*	33-46
MERG	a- wave	amplitude (μV)	24.9 ± 4.7	22.0 ± 3.2*	19.3 ±4.0**	26-62
		latency (ms)	15.7 ± 2.3	19.6 ± 2.0*	25.1 ± 4.1**	13-16
	b-wave	amplitude (μV)	100.3 ± 6.1	61.2 ± 5.2**	34.7 ± 2.6***	103-250
		latency (ms)	32.1 ± 1.9	34.2 ± 6.0*	43.7 ± 4.0*	29-33
RERG	amplitude N1– P1 (μV)		53.3±3.5 *	40.1±4.3**	20.9±3.6**	57-223

* - p < 0.05; ** - p < 0.01; ***- p < 0.001 –statistically significant difference in relation to the norm

In the PSCA in the patients' group II, Vmax value corresponded to average of 6.2±0.001 (p<0.001), and in group III – 5.0±0.8 (p<0.001). Vmin values in PSCA in group II were equal to average of 3.1±0.05 (p<0.01), in group III – 2.6±0.01 (p<0,001). RIs of this vessel were also statistically significantly reduced to 0.52±0.01 with p<0.001 in group II, and to 0.47±0.02 with p < 0.001 in group III (Schedule 1).

Changes in ERG indicators were observed in all the studied patients groups, moreover statistically significant violations were registered even in the patients with initial stage of RP.

In accordance to total ERG data, significant disturbances were detected in the groups II and III (Table 2). The amplitude of the a-wave in the patients of group II was reduced to an average of 49.6±8.0 μV, and in patients group III – 15.3±7.1 μV. The amplitude of the b-wave in patients group II was reduced to average of 114.4±8.2 μV, and in patients group III – 40.1±7.0 μV. These changes were significant with p<0.001. In the patient group I, the amplitudes of a- and b-waves had statistical confidence values of p<0.05. The latency of a-wave in patients group II increased to an average of 22.8±4.2 msec (p<0.05), and in the patients group III – to 23.5± 4.0 msec (p<0.05). The latency of the b-wave in patients group II corresponded to 44.7±2.3 msec, which is statistically non-signifant change, and in patients group III it was increased to an average of 51.0±2.2 msec (p<0.01).

MERG indicators were impaired in the patients of groups II and III (Table 2). No statistically significant changes were observed in the patients of group I. The amplitude of the a-wave in the patients of group II was reduced to an average of 22.0±3.2 μV (p<0.05), and in the patient group III – to 19.3±4.0 μV (p<0.01). The amplitude of the b-wave in patients group II was reduced to an average of 61.2±5.2 μV (p < 0.01), and in the patient group III – to 34.7±2.6 μV (p<0,001). The latency of the a-wave in the patient group II was extended to an average of 19.6± .0 msec (p<0.05), and in the patient group III – to 25.1±4.1 msec (p<0.01). The latency of the b-wave in the patient group II corresponded to an average of 34.2±6.0 msec (p<0.05), and in the patient group III – 43.7±4.0 msec (p<0.05).

The N1– P1 amplitudes of RERG (Schedule 2) were statistically significantly reduced in all the 3 patients groups. In group I, these indicators corresponded to an average of 53.3 ± 3.5 μV ($p < 0,05$), in group II – 32.0±4.8 μV ($p < 0.01$), and in group III – 20.9 ± 3.6 μV ($p <0.01$).

The results of our research are consistent with the results of other authors (Steuer et al., 2005; Maquire et al., 1996; Akyol et al., 1995; Cellini et al., 2010). In these studies, hemodynamic parameters in the eye vessels were studied through CDI and the conclusion was made, that there was insufficient blood supply to outer and middle layers of the retina. The authors note significant decrease in the velocity of blood flow in the CRA and PSCA, Vmax and Vmin indicators, and a decrease of the resistance index in both vessels. Changes in hemodynamic parameters in the mentioned studies are consistent with the results of our study.

Based on the correlation analysis of the results, the interrelation between some hemodynamic parameters and ERG indicators was established. Direct correlation between Vmin and Vmax values in CRA and amplitudes of b-wave of total ERG (r=0.53; r=0.46, correspondingly), as well as between Vmax and Vmin values in PSCA and amplitudes of a-wave of total ERG (r = 0.80; r = 0.82, respectively) was found. Reverse correlation between Vmin and Vmax values in CRA and latency of b-waves of total ERG (r=-0.45; r=-0.47, correspondently), as well between Vmax and Vmin values in PSCA and latency of a-wave of total ERG (r=-0.47; r=-0.39, respectively) was found. Detected changes are statistically significant.

In the studies of Dolan and colleagues (2002), Seeliger and colleagues (1998), Marmor and colleagues (2009) the level of changes in functional status of central retina was analysed and studied, however, no studies were implemented depending on the stages of RP. Kisel'eva and colleagues (2015) studied the characteristics of the eye hemodynamics and retina electrogenesis in the adult patients group, but on smaller clinical material. The obtained results are in accordance with our own. In the work of Zhang and colleagues (2013) an interrelation between decreased blood flow of chorioretinal complex and impaired function of the eye photoreceptors based on MRI and ERG data was also established. However, there is no a correlation analysis and study of statistical reliability of the changes, implemented in the work.

CONCLUSION

The results of ERG and CDI show the presence of statistically significant changes in the cases of medium severe and severe stages of RP, as well indicate to advent of disturbances already in the initial stages. CDI of the patients with RP allows detecting timely hemodynamic disorders. Analysis of ERG data indicates the suppression of photoreceptors function in parallel to the disorders of blood flow in the eye vessels.

Thus, it is necessary to conduct EPS and dopplerographic studies for early diagnosis and monitoring of the patients with RP, as well for implementation of therapeutic and preventive measures.

REFERENCES

Гашимова Н.Ф., Насруллаева М.М., Мамедова Т.М., Бабаева Л.А., Мирзоева Ф.Х. (2010) Вопросы патогенеза, классификации и лечения тапеторетинальных абиотрофий (обзор литературы). *Офтальмология* (Баку), **№4:** 87-91.

Касимов Э.М., Мамедзаде А.Н. (2012) Состояние гемодинамики глаза при пигментном ретините у взрослых пациентов. *Офтальмология* (Баку), **№1(8):** 88-91.

Киселева Т.Н., Зольникова И.В., Деменкова О.Н., Рамазанова К.А. и др. (2015) Особенности гемодинамики глаза и электрогенеза сетчатки при пигментном ретините. *Вестник офтальмологии* (Москва), **№5:** с.14-19.

Шамшинова А.М. (2001) Наследственные и врожденные заболевания сетчатки и зрительного нерва. Москва: 528 с.

Щуко А.Г., Малышев В.В., Жукова С.И. (2010) Пигментная абиотрофия сетчатки. Москва: 112 с.

Akyol N., Kükner S., Celiker U., Koyu H., Lüleci C. (1995) Decreased retinal blood flow in retinitis pigmentosa. *Can J. Ophthalmol*., **30(1):** 28-32.

Cellini M., Strobbe E., Gizzi C., Campos E.C. (2010) ET-1 plasma levels and ocular blood flow in retinitis pigmentosa. *Can. J. Physiol. Pharmacol*., **88(6):** 630-635.

Dolan F., Parks S., Hammer H., Keating D. (2002) The wide field multifocal electro-retinogram reveals retinal dysfunction in early retinitis pigmentosa. *Brit. J. of Ophthalmol.*, **86 (4):** p.480-481.

Eden M., Payne K., Combos R., Hall G., McAllister M. et al. (2013) Valuing the benefits of genetic testing for retinitis pigmentosa: a pilot application of the contingent valuation method. *Brit. J. of Ophthalmol.*, **97(8):** 1051-1056.

Hamblion E.L., Moore A.T., Jugnoo S. (2010) Incidence and patterns of detection and management of childhood-onset hereditary retinal disorders in the UK. *Brit. J. Ophthalmol*., **№20**, p.1178.

Maguire A.M., Grunwald J.E., Dupont J. (1996) Retinal hemodynamics in retinitis pigmentosa. *Am. J. Ophthalmol*., **122 (4):** 502-508.

Marmor M.F., Fulton F.B., Holder G.E., Myake Y. et al. (2009) İS-CEV standard for full-field clinical electroretinography (2008 update). *Doc. Ophthalmol*., **118(1):** 69-77.

Parodi M., Cicinelli M., Rabiolo A., Pierro L., Gagliardi M. Et al. (2017) Vessel density analysis in patients with retinitis pigmentosa by means of optical coherence tomography angiography. *Brit. J. of Ophthalmol.*, **101 (4):** 428-432.

Riera M., Abad-Morales V., Navarro R., Ruiz-Nogales S., Mendez-Vendrell P. et al. (2019) Expanding the retinal phenotype of RP1: from retinitis pigmentosa to a novel and singular macular dystrophy. *Brit. J. of Ophthalmol.*, **101 (8):** 313-318.

Robson A., Saihan Z., Jenkins S., Fitzke F., Bird A et al. (2006) Functional characterization and serial imaging of abnormal fundus autofluortescence in patients with retinitis pigmentosa and normal visual acuity. *Brit. J. of Ophthalmol.*, **90 (4):** 472-479.

Sandberg A., Rosner B., Weigel-DiFranco C., Berson E. (2011) Lack of scientific rationale for use of valproic acid for retinitis pigmentosa. *Brit. J. of Ophthalmol.*, **95 (5):** 428-432.

Seeliger M., Kretschmann U., Apfelstedt-Sylla E., Ruther K., Zrenner E. (1998) Multifocal electroretinography in retinitis pigmentosa. *Am. J. of Ophthalmol.*, 1998, **125 (2)**, p.214-226.

Steuer E., Formińska-Kapuścik M., Kamińska-Olechnowicz B. et al. (2005) Assessment of blood flow in retinal pigment degeneration. *Klin. Oczna*., **107 (1-3):** 57-59.

Zhang Y., Harrison J., Nateras O., Chalfin S. (2013) Decreased retinal-choroidal blood flow in retinitis pigmentosa as measured by MRI. *Doc. Ophthalmol.*, **126 (3):** 187-197.

Pigmentli retinit zamanı cavan yaşlı pasiyentlərdə doppleroqrafik və elektrofizioloji tədqiqatlar

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İrsi xəstəliklər tibbin digər sahələrində olduğu kimi, oftalmologiyanın ağır patologiyalarından sayılır. PR tor qişanın ümumi irsi distrofiyasıdır. Bu xəstəlik görmə funksiyalarının itməyinə və korluğa gətirib çıxarır. İşin məqsədi cavan yaşlı pasiyentlərdə PR-in müxtəlif mərhələləri zamanı göz damarlarında hemodinamik parametrlərin və ERQ göstəricilərinin dəyişikliklərinin korrelyasiyasının öyrənilməsi olmuşdur. PR diaqnozu ilə yaş həddi 15-24 arasında olan 100 pasiyentdə (200 göz) müayinə aparılmışdır. Onlardan 41-i qadın, 59-u kişi olmuşdur. PR zamanı funksional dəyişikliklərə görə pasiyentlər 3 qrupa ayrılmışdır: I qrup – başlanğıc mərhələlı pasiyentlər 31 nəfər (62 göz), II qrup – orta ağır mərhələli pasiyentlər 40 nəfər (80 göz), III qrup – ağır mərhələli pasiyentlər 29 nəfər (58 göz). Bütün xəstələrdə gözdə qan axınını

öyrənmək üçün RDK «TOSHIBA» firmasının «Nemio XG SSA-580A» ultrasəs diaqnostik aparatında 8 mHz xətti datçik vasitəsilə həyata keçirilmişdir. Həmçinin ROLAND CONSULT Super Color Ganz feld Q450 SC (Almaniya) aparatında ERQ (ümumi, ritmiki və makulyar) aparılmışdır. ERG və RDK nəticələri PR-in orta ağır və ağır mərhələlərində statistik dürüst dəyişikliklərin olduğunu göstərir, həmçinin başlanğıc mərhələdə də pozğunluqların olduğunu bildirir. RDK PR-də hemodinamik pozğunluqları zamanında aşkarlamağa imkan yaradır. ERG göstəricilərinin təhlili fotoreseptorların funksiyasının azalması göz damarlarında qan axınının pozulmasına paralel baş verdiyini bildirir. Beləliklə, PR zamanı pasiyentlərin erkən diaqnostikasında və monitorinqində, eləcə də müalicə-profilaktik tədbirlərin aparılması üçün dopplerоqrafik və elektrofizioloji müayinələrin aparılması zəruridir.

***Açar sözlər**: Pigmentli retinit (PR), rəngli doppler kartlaşdırılma (RDK), elektroretinoqrafiya (ERQ)*

Допплерографические и электрофизиологические исследования при пигментном ретините у лиц молодого возраста

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Одними из тяжелых патологий в офтальмологии, как и в других отраслях медицины, являются наследственные болезни. ПР представляет собой наследственную генерализованную дистрофию сетчатки. Эта патология приводит к потере зрения вплоть до слепоты. Цель работы – изучить изменения гемодинамических параметров в сосудах глаза и показателей ЭРГ и провести корреляционный анализ у пациентов молодого возраста с различными стадиями ПР**.** Обследованы 100 пациентов (200 глаз) с пигментным ретинитом в возрасте 15-24 лет: из них - 41 пациент женского пола, 59 – мужского пола. Пациенты были распределены на 3 группы. В I группу был включен 31 пациент (62 глаза) с начальной стадией ПР. Во II группу было включено 40 пациентов (80 глаз) со среднетяжелой стадией ПР. В III группу было включено 29 пациентов (58 глаз) с тяжелой стадией ПР. Всем пациентам проводилась ЭРГ – общая, ритмическая - 30 Гц (РЭРГ), макулярная (МЭРГ) – на аппарате ROLAND CONSULT Super Color Ganz feld Q450 SC (Германия). Ультразвуковые исследования включали В-сканирование глазного яблока и допплерографию сосудов глаза методом ЦДК. Исследовались глазная артерия (ГА), центральная артерия сетчатки (ЦАС) и задние короткие цилиарные артерии (ЗКЦА). Результаты ЭРГ и ЦДК показывают наличие статистически достоверных изменений при среднетяжелых и тяжелых стадиях ПР, а также указывают на наличие нарушений уже на начальных стадиях. ЦДК у пациентов с ПР позволяет своевременно выявить гемодинамические нарушения. Анализ данных ЭРГ указывает на угнетение функции фоторецепторов параллельно нарушениям кровотока в сосудах глаза. Таким образом, необходимо проведение электрофизиологических и допплерографических исследований для ранней диагностики и мониторинга пациентов с ПР, а также для проведения лечебно-профилактических мер.

***Ключевые слова:** Пигментный ретинит (ПР), цветовое допплеровское картирование (ЦДК), электроретинография (ЭРГ)*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 66-88 (2020)

Neurodegeneration as mitochondrial pathology: Signaling mechanisms and new routes for life-time diagnostics and targeted therapy (review)

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Accepted for publication: 24 April 2020

Structural and functional alterations of mitochondria have been shown to be responsible for a wide variety of clinical disorders that are referred to as "mitochondrial diseases". It is now obvious that many factors are involved in transport of mitochondrial proteins including cytokines, chaperones, chemokines, neurosteroids, ubiquitin and many others. At the same time, the participation and the role of biogenic amines and peptide hormones (which are produced by the diffuse neuroendocrine system cells located in different organs) in endogenous mechanisms of mitochondrial diseases are still unknown. Taking into account the wide spectrum of biological effects of biogenic amines and peptide hormones, and especially their regulatory role for intracellular communication, it seems important to analyze the possible participation of these molecules in the pathogenesis of mitochondrial disorders as well as to draw up a new way for elaboration of a new markers for lifetime diagnosis, definition of prognosis and efficiency of specific therapy in neurodegenerative diseases.

Keywords: *Mitochondrial diseases, biogenic amines, peptide hormones, diffuse neuroimmunoendocrine system, lifetime diagnostics, neurodegeneration*

INTRODUCTION

Mitochondria are a key beachhead of cell pathology

Mitochondria are the sites of crucial cellular functions in eukaryotic cells responsible of converting energy derived from chemical fuels by harnessing this energy for biological purposes through a chemiosmotic coupling. Moreover, mitochondria have also been suggested as an important source of cellular second-messenger molecules (reactive oxygen intermediates and others), which are involved in many gene regulatory pathways. In metabolically active cells, mitochondria are the most abundant organelles, and up to 10-20% of the total intracellular proteins have been estimated to be present within this organelle (Devine and Kittler, 2018).

doi.org/10.29228/jlsb.44

In spite of mitochondria having their own DNA encoding mt tRNA, mt rRNA and several polypeptides, they import virtually all of their proteins from the cytoplasm. This import process faces the challenge to route the proteins to their correct submitochondrial compartment, and this process required that most of them must be transported across two membranes. This challenge is met by the joint action of two distinct protein transport systems, one in the outer membrane and the other in the inner membrane.

Primary defects in mitochondrial function are involved in over 100 diseases, and the list continues to grow. Structural and functional alterations of mitochondria have been shown to be responsible for a wide variety of clinical disorders that are referred to as "*mitochondrial diseases*" or "*mitochondrial cytopathies*" (Schapira, 1993; Luft, 1994). It has become apparent that genetic defects in the synthesis of mitochondrial proteins may be the underlying cause of diseases affecting organ systems including the nervous system, skeletal and cardiac muscle, liver and others.

It is now obvious that many factors are involved in transport of mitochondrial proteins including cytokines, chaperones, chemokines, neurosteroids, ubiquitin and many others (Mihara and Omura, 1996; Kroemer et al., 1998; Gale and McColl, 1999). At the same time the participation and the role of biogenic amines and peptide hormones (highly active biologically substances, which are produced by neuroendocrine cells located in different organs) in endogenous mechanisms of mitochondrial diseases are still unknown.

Taking into account the wide spectrum of biological effects of biogenic amines and peptide hormones, and especially their regulatory role for intracellular communication, it seems important to analyze the possible participation of these molecules in the pathogenesis of mitochondrial disorders, especially for neurodegenerative diseases.

The aim of this paper is to analyze the possible role of regulatory peptides and related molecules in endogenous mechanisms of neurodegenerative processes and to identify avenues for further research.

Diffuse neuroimmunoendocrine system: Evolution of knowledge

In last two decades there are more and more evidences that identical peptide hormones and biogenic amines are synthesized by different cells having neuronal, immune or endocrine assignment. Historically, Pearse was the first who in the late 1960's suggested that a specialized, highly organized cell system should exist in organisms, whose main feature was the ability of component cells to produce peptide hormones and biogenic amines.

His concept was based on an extensive series of experiments for distinguishing endocrine cells in different organs, identifying endocrine cell-generated products and performing a thorough cytochemical and ultrastructural analysis of these cells.

Pearse has obtained that a variety of cell types, widely dispersed throughout the organism, have a common ability of absorbing monoamine precursors (5-hydroxytryptophan and L-dihydroxyphenylalanine) and decarboxylating them, thus producing biogenic amines. That ability accounts for the term APUD, an abbreviation of "Amine Precursor Uptake and Decarboxylation" used by Pearse to designate this cell series (Devine and Kittler, 2018; Phu et al., 2020).

To date, the APUD series includes over 60 types of endocrine cells located in gut, pancreas, urogenital tract, airway epithelium, pineal gland, thyroid gland, adrenals, adenohypophysis and hypothalamus, carotid body, skin, sympathetic ganglia, thymus, placenta and other organs. Meanwhile the advent of radioimmunological methods and the rapid development of immunohistochemistry resulted in the establishment of a completely unexpected phenomenon, i.e., the same biogenic amines and peptide hormones were identified in neurons and endocrine cells. Just in this year 25 years have passed since Roger Guillemin has been awarded by Nobel Prize and presented his Nobel Prize Lecture entitled "Peptides in the brain. New endocrinology of the neuron" (Ramage et al., 1993).

The accumulated data did not fit the traditional concepts of hierarchical dependence within two main regulatory systems, viz., the nervous and en-

docrine systems. It became more and more data that the mechanism of biological regulation should be based on the coordinated functional interaction between the endocrine system and the central and peripheral nervous system considering the common type of information perception and transmission at subcellular, cellular and tissue levels.

Many studies on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones, and locally or remotely as hormones within the endocrine system, enables both system to be incorporated into the universal ***diffuse neuroendocrine system (DNES)*** (Hernandez et al., 1999a)**.** Actually, it should be possible to unite in the organisms the structurally isolated nervous and endocrine systems by means of functional relationships between biogenic amines and regulatory peptides and, to a certain extent, to provide a basis for the concept of integrated functions. Located in practically all organs and producing biologically active substances, the DNES cells play role of regulators of homeostasis acting via neurocrine, endocrine and paracrine mechanisms (Hernandez et al, 1999b).

Later it was shown that the nervous and immune systems have well-established and very closed related interrelations for regulate systemic homeostasis that involves the production and secretion of a variety of cellular mediators known as ***regulatory peptides*** (peptide hormones, cytokines, chemokines, integrins and others) (Schapira, 1993). Peptide hormones, cytokines and other related molecules regulate homeostasis in the tissue of origin, either via local actions or by recruitment of external systems that facilitate restoration of local homeostasis.

The studies on isolated-cell systems have confirmed that many regulatory peptides and biogenic amines are expressed within the brain. There are many peptidergic neurons and glial cells in the brain which can produce peptide hormones and biogenic amines; also besides neurons, immune cells, such as macrophages, T-lymphocytes, eosinophilic leukocytes and mast cells, which invade the brain after injury or inflammation, are a rich source of cytokines and other active molecules (Luft, 1994; Mihara and Omura, 1996; Molnar and Kovaes, 2017).

Such chemical common character of three regulatory systems, namely nervous, endocrine and immune systems stimulated the development of new research field called ***neuroimmunoendocrinology*** which mainly studied the mutual interrelationships between these regulatory systems (Kroemer et al., 1998). It seems to be necessary to underline that numerous investigations in this field of study fail to take one phenomenon into account which we consider as very important fact.

There is a following circumstance - **the nervous and immune cells together with APUD cells represent in most visceral organs, where they are available to produce many peptides and biogenic amines which are identical to the same in the brain and central organs of immune and endocrine systems**.

Therefore the close interrelations between three regulatory systems provide with anatomical/functional property - immune and nervous system have their representation in visceral organs through the peptidergic/aminergic neurons (and/or nerve fibers) as well as through the immunocompetent cells producing different peptide molecules; in its turn, the endocrine system represents in central nervous system and immune organs through APUD cells (e.g. hypothalamic neurosecretory cells and others).

Thus, obviously that cell types of all three classical regulatory systems (nervous, endocrine and immune) represent in each visceral organ, including the central organs of homeostatic regulation (e.g. brain, thymus, thyroid, etc).

Hence it follows to be possible to unite peptidergic/aminergic neurons, APUD cells and peptide-producing immunocompetent cells into a single common functional system and to extend the term ***diffuse neuroendocrine system (DNES)*** to the new term ***diffuse neuroimmunoendocrine system (DNIES)***.

Exactly the DNIES is a field of the study for neuroimmunoendocrinology as a new scientific biomedical discipline which integrates our knowledges about signalling mechanisms of homeostatic regulation.

Neuropathology of Alzheimer's and Parkinson's diseases

The most important diseases among all mitochondrial disorders are the neurodegenerative

diseases, including most notably Alzheimer's disease (AD) and Parkinson's disease (PD). AD is characterized by a progressive loss of memory, resulting in dementia and death. AD affects over 20 million people worldwide and its incidence is expected to double over the next 30 years (Price, 1999).

A triad of neuromorphophysiological features characterize AD and include amyloid-β plaques (senile plaques), neurofibrillary tangles and extensive neural loss particularly in the hippocampus and cerebral cortex (Dickson, 1997); these changes are associated with dementia and characteristic neurobehavioral consequences. The signs of the disease differ among individuals with the majority of cases arising sporadically and commonly they have a late life onset (after 65 years of age); in a less common form of familial AD, the onset of the condition is typically much earlier (40-50 years of age) (Price, 1999).

Parkinson's disease (PD) is a major neurodegenerative disorder with a prevalence of roughly 150 cases for every 100,000 elderly people. The condition is characterized by the progressive deterioration of the dopamine containing neurons in the pars compacta of the substantia nigra in the brain stem; the loss of these catecholaminergic neurons is associated with a variety of sensory and motor impairments which lead to tremor, rigidity and akinesia (Li and Song, 2020).

For an individual to manifest signs of PD it is estimated that the nigro-striatal dopaminergic neuronal population must be depleted by at least 80%. Thus, in most cases the initiating factor for PD probably precedes the overt signs of parkinsonism by 5-10 years (Li and Song, 2020).

Pathogenesis of neurodegenerative diseases: Related molecules and cellular bases. Cytokines

Major cytokines for brain function are neurotrophins (BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor and GDNF, glial-derived neurotrophic factor) and neuropoietins (especially interleukin-6, IL-6). They participate in the mechanisms of growth and differentiation of neurons and in neurotransmission (Merrill and Jonakait., 1995). The most abundant source of cytokines, particularly after local damage, appears to be activated microglia, although neurons, astroglia, perivascular and endothelial cells can also express cytokines (Kunkl et al., 2020). Studies on the localization and expression of peptide hormones and cytokines in response to specific stimuli have important implications for their actions in the CNS.

For example, it is clear now that cytokine expression is upregulated rapidly in situations of tissue stress, and that cytokines have important actions that are consistent with their role in restoration of tissue homeostasis. Cytokines have been reported to influence many central neurotransmitters, including noradrenaline, serotonin, GABA and expression of a number of neuropeptides (somatostatin, substance P, opioids, VIP, etc.) in several brain regions (Neumann and Wekerle, 1998). However, the interrelationships between each of these varied neurotransmitter responses and their relevance to specific cytokine actions have yet to be defined.

Similarly, a number of second messenger systems in neurons are affected not only by cytokines, but melatonin and other hormones and mediators, including activation of cAMP, increased activity of protein kinase C, synthesis of nitric oxide, release of arachidonic acid and Ca^{2+} flux (Mrak et al., 1997; Borjigin and Snyder, 1999). Thus, now it seems likely that the behavior of practically all molecules involved in pathogenesis of AD and other mitochondrial diseases may be under control of regulatory peptides.

Several cytokines have been reported to influence neuronal differentiation and growth as well as to acutely modify synaptic plasticity in brain slice preparations. For many cytokines and other peptide molecules, conflicting data exist, indicating that many can exert neurotrophic, neuroprotective and neurotoxic actions. As reported, transgenic mice overexpressing IL-6 in astrocytes show marked neurodegeneration, and inhibition of action of IL-1 and IL-6 markedly inhibits the neurodegenerative processes (Griffin et al., 1995). IL-1 induces expression of β-amyloid precursor protein (β-APP) and adhesion molecules in neural tissue (Morganti-Kossmann et al., 1992).

Many hormones influence cytokine actions: glucocorticoids are potent inhibitors of the synthesis and actions of cytokines; also, melanocyte stimulating hormone and vasopressin attenuate actions of cytokines in the brain, and these peptides, as well as lipocortin, have been implicated in impaired febrile responses to cytokines in ageing animals (Rothwell and Hopkins, 1995).

The pathological presentation of AD, the leading cause of senile dementia, involves regionalized neuronal death and an accumulation of intracellular and extracellular filamentous protein aggregates which form lesions termed neurofibrillary tangles and senile plaques, respectively (Dickson, 1997). Several independent parameters have been suggested as the primary factor responsible for this pathogenesis, including apolipoprotein ε genotype, hyperphosphorylation of cytoskeletal proteins, or metabolism of amyloid β.

Amyloid β (Aβ). The view that a relationship exists between amyloid deposits and neurofibrillary lesions remains an important unresolved issue in our understanding of the pathogenesis of AD (Auld et al., 1998; Selkoe, 1998; Iadanza et al., 2018).

Amyloid plaques (AP), which are a classical neuropathological characteristic of AD, have been reproduced in transgenic mice. These mice exhibit selective neuronal death in the brain regions that are most affected in AD, suggesting that AP formation is directly involved in AD neuron loss (Calhoun et al., 1998). On the other hand, non-Aβ component of AD amyloid (NAC) is the second component in the amyloid from brain tissue of patients afflicted with AD (Ueda et al., 1993).

Its precursor protein (NACP) was shown to be a brain-specific protein. NACP was more abundant in the neocortex, hippocampus, olfactory bulb, striatum, thalamus and cerebellum. Confocal laser microscopic analysis revealed that anti-NACP immunostaining was colocalized with synaptophysin - immunoreactive presynaptic terminals, therefore NACP is a synaptic protein, suggesting that synaptic aberration observed in senile plaques might be involved in amyloidogenesis in AD (Iwai et al., 1995).

Tau-protein. There are some data indicating that even small numbers of neurofibrillary lesions are pathological and may represent the early stages of AD. There are also many neurodegenerative diseases with numerous positive filamentous lesions: AD, Parkinson's disease (PD), Down syndrome, myotonic dystrophy, and others.

Tau is a microtubule-associated protein that is involved in microtubule assembly and stabilization (Gao et al., 2018). In adult human brain, six isoforms of tau are expressed, which are produced by alternative splicing of mRNA from a single gene located on the long arm of chromosome 17. Tau protein mRNA is expressed predominantly in neurons, with recent reports indicating its additional presence in oligodendrocytes.

Within nerve cells tau protein is present mainly in axons (Congdon and Sigurdsson, 2018). In some recent studies the expression of tau-protein has also been shown in cultured skin fibroblasts from Alzheimer's disease patients (Blass et al., 1991). A number of studies have characterized tau filaments in various diseases by electron microscopy and immuno-electron microscopy (Spillantini and Goedert, 1998). Currently, three types of filament morphologies can be distinguished and they have a diagnostic significance (for example, type I more often can be identified in AD, type II more characteristic feature for PD).

Tau pathology is one of the central neuropathological characteristic of a number of neurodegenerative disorders since the events leading to the formation of tau filaments are sufficient to produce nerve cell degeneration (Lee and Trojanowski, 1999). Therefore, an important direction for further study is to find either endogenous and exogenous ways to prevent tau filament formation. In this connection, one of the possible ways to prevent tau filament formation and development of amyloid plaque could be to identify the endogenous mechanisms of interpeptide communications.

It appears that biologically active substances (neuropeptides, cytokines, etc.) have several, probably distinctive, actions on the nervous system: as communicators to the brain of systemic injury and other disorders; as modulators of brain responses to peripheral organs; as neuromodulators and neurotransmitters of the CNS control of systemic host defence responses to disease and injury; and as molecules that inhibit or mediate neurodegeneration and repair in the brain. The relevance of peptide molecules and related protein actions to

a variety of neurological disorders is now being determined, and has opened a potentially fruitful area of research and therapeutic development.

Synuclein proteins. Several mitochondrial neurodegenerative disorders are characterized by intracellular protein accumulations or inclusions, such as the Lewy bodies (LB) in PD. α-synuclein is a proposed component of LB. It was shown immunohistochemically, that indeed LB in brains of sporadic PD patients are strikingly synuclein-positive (Spillantini et al., 1997). In addition to synuclein, LB contain ubiquitin, ubiquitin C-terminal hydrolase, and proteasomal subunits, major components of the cellular protein degradation pathway (Alves-Rodrigues et al., 1998; Pallares-Trujillo et al., 1998). The following areas of the brain are often involved in this pathological process: the striatum (putamen), substantia nigra, locus coeruleus, inferior olive, pons, and cerebellum.

Synuclein proteins are produced by three genes (Clayton and George, 1998). They share a structural resemblance to apolipoproteins. α-synuclein is distinguishable from the other synucleins. It uniquely has a histidine at residue 50 (β has a unique histidine at 65). Recent reports of synuclein immunoreactivity in LB suggest the presence of α but not β synuclein (Clayton and George., 1998). The structure, function and localization of the synucleins might be subject to regulation by signals associated with synaptic activity and neuritic growth.

In general, the distribution of α-synuclein in the brain is similar to the distribution of brain pathology in AD (Alves-Rodrigues et al., 1998). Additional portions of the synuclein protein are present in amyloid plaques in AD. A significant increase in cytosolic synuclein immunoreactivity in frontal cortical extracts in early AD cases was reported; it seems possible that α-synuclein might potentiate the long-term development of AD (Dehghani et al., 2020).

Chemokines. Chemokines and chemokine receptors in the CNS are constitutively expressed at low levels in astrocytes, microglia and neurons of the developing and adult brain and they are induced by inflammatory mediators (Mennicken et al., 1999). Furthermore, chemokines and their receptors are upregulated in various neuropathology including brain tumours and AD (Asensio and Campbell, 1999). Cell culture studies support a role for chemokines in the differentiation and migration of brain cells.

For example, IL-8 enhances the survival of neurons and the number of microglial and astroglial cells in rat hippocampal cultures, and it influences neuronal growth in the human brain (Asensio and Campbell 1999). Chemokines also modulate angiogenesis or neovascularization in lesion brain areas. Moreover, an upregulation of the CXCR2 protein (receptor for IL-8) occurs in senile plaques adjacent to the hippocampus in the brains of AD patients. Because IL-8 promotes survival of hippocampal neurons, a possible involvement of IL-8/CXCR2 in compensatory and reparative mechanisms in the Alzheimer's brain should be considered (Mennicken et al., 1999).

Integrins. Integrins are the major family of cell surface receptors that mediate attachment to the extracellular matrix, and specific classes of integrins also mediate important cell-cell adhesive interactions. These integrin-mediated adhesive interactions are intimately involved in the regulation of many cellular functions, including embryonic development, tumor cell growth, programmed cell death, hemostasis and many others (Clark and Brugge, 1995).

It appears that multiple receptor systems can synergize with integrins to regulate cell proliferation, motility, secretion, and other cellular events. The signalling proteins activated by these synergistic agents are common to many receptor pathways. Thus, although unique pathways may be activated by individual classes of receptors, cross talk between integrins and other receptor pathways is critically involved in the integration of signals that converge on cells in their natural environments *in vivo*.

Chaperones. Molecular chaperones (Hsp28, αB-crystallin) are also involved in AD (Martinus et al., 1995). Detailed insights into the role of molecular chaperones have come from studies of mitochondrial protein biogenesis, a process in which chaperones act as unfoldases, pulling devices, and foldases. One of the chaperones is mitochondrial import stimulation factor (MSF) (Hachiya et al., 1993). It seems this factor is a

conformational modulator of mitochondrial precursor proteins. Other studies showed that some heat shock proteins (Ssa1p, Ssa2p and especially Hsp70) are involved in the import of proteins into mitochondria, as well as into the endoplasmic reticulum and nuclei (Kang et al., 1990; Lithgow et al., 1993).

Neurosteroids. It is now established that the brain itself also synthesizes steroids *de novo* from cholesterol in a variety of vertebrates (Paul and Purdy, 1992). In the brain, glial cells play a major role in neurosteroid formation and metabolism (Kreutzberg, 1996). Purkinje cells produce neurosteroids (pregnenolone and progesterone). These cells demonstrate immunopositive staining with antibody to key steroidogenic enzyme cytochrome P450scc (Tsutsui and Ukena, 1999).

Progesterone (one of the main neurosteroids) is shown to be produced from pregnenolone by Schwann cells in peripheral nerves, and some observations indicate a role for locally produced progesterone in myelination, demonstrate that progesterone is not simply a sex steroid, and suggest a new therapeutic approach to promote myelin repair (Koeniget al., 1995). Mitochondria of C6-2B glioma cell line participate in the biosynthesis of pregnenolone converting (22R)-22-hydroxycholesterol to pregnenolone by a mechanism blocked by aminoglutethimide (Papadopoulos et al., 1992).

Oxygen radicals. Many diseases related to aging may involve oxygen radicals at some stage in their development. In these diseases, it has been proposed that mutations of mthDNA and changes in cellular bioenergetics contribute in some way to the aging process and to the development of degenerative diseases (Shigenaga et al., 1994).

Though only recently uncovered as a physiologic messenger, nitric oxide (NO) is increasingly appreciated as a major regulator in the nervous, immune, and cardiovascular systems (Nathan, 1992). Besides mediating normal functions, NO has been implicated in many different pathophysiologic states including neurodegenerative diseases (Ames et al., 1993).

Of all the organs in the body, the central nervous system (CNS) takes more than its share of oxidative abuse (Bolanos et al., 1997). The reasons for this are several-fold. The brain although constituting only a small percentage (in the human about 2%) of the body weight consumes a disproportionately large amount (in the human about 20%) of the O_2 inhaled. Given that by-products of O_2 are toxic, it is not suprising that neural tissue may thus be destroyed at a more rapid rate than other organs.

Mitchondrial DNA (mtDNA) has more than 10 times the level of oxidative DNA damage than does nuclear DNA (Ames et al., 1993). This increase may be due to a lack of mtDNA repair enzymes, a lack of histones protecting mtDNA, and the proximity of mtDNA to oxidants generated during oxidative phosphorylation. The cell defends itself against this high rate of damage by a constant turnover of mitochondria, thus presumably removing the damaged mitochondria that produce increased oxidants.

Despite this turnover, oxidative lesions appear to accumulate with age in mtDNA at a higher rate than in nuclear DNA. Oxidative damage could also account for the mutations in mtDNA that accumulate with age (Smith et al., 1995).

That oxidative stress may be a culprit in neuronal loss in AD has been emphasized in recent years and the evidence is becoming progressively stronger that radicals are involved in the neural pathogenesis of AD (Reiter, 1995). The free radicals that have been incriminated as causing neuronal loss are believed to be generated by Aβ (Smith et al., 1995).

According to the free radical theory of PD, dopaminergic neurons are lost as a consequence of their relatively high exposure to reactive oxygen species, most notably H_2O_2 which is produced during both the enzymatic, via monoamine oxidase activity, and non-enzymatic, due to the autooxidation, destruction of dopamine (Fahn and Cohen, 1992).

Not only does oxidative stress destroy the dopaminergic neurons but it also compromises mitochondrial oxidative phosphorylation leading to decreased energy output by these organelles and eventually to secondary death of the cells.

Glutamate. There is an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders (Reiter, 1998).

At the same time, excessive or persistent activation of glutamate/gated ion channels may cause neuronal degeneration in these same conditions. Glutamate and related acidic amino acids are thought to be the major excitatory neurotransmitters in brain and may be utilized by 40 percent of the synapses (Coyle and Puttfarcken, 1993). Thus, two broad mechanisms, oxidative stress and excessive activation of glutamate receptors, are converging and represent sequential as well as interacting processes that provide a final common pathway for cell vulnerability in the brain.

The broad distribution in brain of processes regulating oxidative stress and mediating glutamatergic neurotransmission may explain the wide range of disorders in which both have been implicated. Yet differential expression of components of the processes in particular neuronal systems may account for selective neurodegeneration in certain disorders.

Although NO participates in normal synaptic transmission, excess levels of NO are neurotoxic. NO stimulates glutamate neurotoxicity which may contribute to dysfunction in neurodegenerative diseases such as Alzheimer's and Huntington's diseases.

Evidence is now emerging that activation of glutamate-gated cation channels may be an important source of oxidative stress and that these two mechanisms may act in a sequential as well as a reinforcing manner, leading to selective neuronal degeneration. Understanding the relation between oxidative stress and glutamate neurotransmission could lead to the development of pharmacologic interventions that disrupt this chain of pathological events without impairing excitatory neurotransmission.

Calcium homeostasis. Brain ageing is associated with a marked decline in mental faculties. One hypothesis postulates that sustained changes in the regulation of intracellular Ca^{2+} concentration are the major cause of neuronal degeneration (Choi, 1995). This "calcium hypothesis" is supported by demonstration of the impairment in aged neurons of molecular cascades that regulate intracellular Ca^{2+} concentration.

The conceptual pillars of this point of view are: dysfunction of intracellular Ca^{2+} homeostasis, and neuronal loss (Verkhratsky and Toescu, 1998). This view of the ageing brain is that the decrease in cognitive function results mainly from neuronal death and that this leads to a decrease in the number of brain cells. Strong support for this hypothesis has come from studies of neurodegenerative diseases, such as AD. In AD there is a profound loss of neurons that correlates well with the decrease in learning abilities and memory function (Dickson, 1997). In addition, a key element of AD pathology, the accumulation of Aβ, has been shown to disrupt neuronal intracellular Ca^{2+} homeostasis (Verkhratsky and Toescu, 1998).

Brain contains a huge population of glial cells that are responsible for the regulation of the brain microenvironment. They can also play an important role in the integrative function of neurons by controlling the concentrations of neurotransmitters and neuromodulators, and thus affecting synaptic transmission. Glial cells, especially astrocytes, rely heavily on neuronal intracellular Ca^{2+} homeostasis, signaling that is involved in most of their response to neurotransmitters (Kreutzberg, 1996).

Apoptosis. In any case, the death of neurons is a final stage of neurodegenerative diseases and this phenomenon is known as apoptosis (Sastry and Rao, 2000). During the development of the vertebrate nervous system, up to 50 percent or more of neurons normally die soon after they form synaptic connections with their target cells (Raff et al., 1993). *Bcl*-2 and related proteins have become a major focus of efforts to unravel the intracellular molecular events that regulate cell survival, and cause cell death (Davies, 1995).

Clarification of the repertoire and functional significance of the interactions between these proteins, and identification of the chain of molecular events in which they fit, will greatly increase our understanding of the apoptotic process. Moreover, neurons are particularly useful for studying the regulation of cell survival and apoptosis because, being postmitotic cells, experimental analysis is not complicated by cell proliferation. Furthermore, the roles of *bcl*-2 related proteins in certain neurons might have important therapeutic implications for neurodegenerative diseases (Hockenbery et al., 1993).

The intracellular membrane-bound protein *bcl*-2 is probably associated with the cytoplasmic surface of the nuclear envelope, endoplasmic reticulum, and mitochondria (Monaghan et al., 1992). Experimental over-expression of *bcl*-2 prevents the death of neurons deprived of particular neurotrophic factors *in vitro*, and rescues developing neurons that would otherwise die *in vivo*.

The intracellular localization of *bcl*-2 to the inner mitochondrial membrane (Hockenbery et al., 1990), endoplasmic reticulum membrane and the nuclear envelope has led to several hypothesis about how it might work. The localization of *bcl*-2 in mitochondria has raised the possibility that it might protect against apoptosis by altering mitochondrial function.

The localization of *bcl*-2 to major sites of oxygen free-radical generation, and evidence that reactive oxygen species might be involved in causing apoptosis in neurons (Jacobson et al., 1993) and other cells have raised the possibility that *bcl*-2 might prevent apoptosis by either acting as an antioxidant or by inhibiting production of free radicals (Fadeel et al., 1999).

Hormones in brain: Localization and role for central nervous functions

Melatonin. During the last decade a great deal of attention has been focused on melatonin, one of the hormones of the DNES, which for many years was considered exclusively as a secretory product of pineal gland (Reiter, 1973; 1992). As soon as highly sensitive antibodies to indolealkylamines became available (Grota and Brown, 1974), melatonin was identified not only in pineal gland, but also in extrapineal tissues, i.e., retina, cerebellum, gut mucosa, airway epithelium, kidney and other tissues (Kvetnoy, 1999) as well as in non-neuroendocrine cells such as mast cells, natural killer cells, eosinophilic leukocytes, platelets and endothelial cells (Kvetnoy, 1999) and in bone marrow cells (Tan et al., 1999).

Also it has now been shown that many cells in different organs possess melatonin receptors and a variety of melatonin receptors have been identified in many areas of human brain (Pang et al., 1993). The above list of cells which contian melatonin indicates that this MT indoleamine has a unique position among the hormones, being found in a variety of organ systems including the CNS.

Functionally, melatonin-producing cells are likely to be part and parcel of the DNES as a universal system of response, control and organismal protection. Taking into account the large number of melatonin-producing cells, the wide spectrum of biological activities of melatonin and especially its properties as a regulator of biological rhythms and antioxidant, extrapineal melatonin may be an important paracrine molecule to ensure optimal cellular function and protection.

The identification of melatonin in pineal gland and in extrapineal tissues stimulated interest in the physiology of this hormone and a wide spectrum of biological activities of melatonin have been uncovered. Some of these functions include the control of biological rhythms, seasonal reproductive events, stimulation of immune processes, cytostatic and antiproliferative effects in vitro and in vivo (Reiter, 1992).

Additionally, another unexpected function of melatonin was uncovered. Thus, the indole has been shown to be a free radical scavenger and antioxidant (Reiter, 1997; Reiter et al, 1993; 1998). Melatonin is now known to be a potent hydroxyl radical scavenger and under some circumstances, it protects against free radical damage more effectively than the well-known scavenger glutathione (Cuzzocrea et al., 1999). Melatonin is now known to scavenge the highly toxic hydroxyl radical, the peroxynitrite anion, singlet oxygen and NO.

Also, secondarily, it reportedly scavenges the superoxide anion radical (Cuzzocrea et al., 1999). Additionally, it stimulates mRNA levels for superoxide dismutase and the activities of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase (all of which are antioxidative enzymes), thereby increasing its antioxidative capacity (Reiter et al., 1998). Also, melatonin inhibits nitric oxide synthase, a pro-oxidative enzyme and stimulates the rate limiting enzyme in glutathione synthesis, α-glutamyl-cysteine synthase (Barlow-Walden et al., 1995).

There is ample evidence that the brain of PD patients exhibits signs of enhanced oxidative stress. Acuna-Castroviejo et al. (Acuna-Castroviejo et al., 1997) have investigated the ability of

melatonin to protect the brain against the toxic effects of MPTP, a drug that produces Parkinson like signs. In this model system, melatonin was strongly protective. Also, Mayo and co-workers (Mayo et al., 1998) assessed the ability of MT to protect against dopamine autoxidation-induced protein damage using the oxygen radical absorbance capacity assay.

The results showed that melatonin reduces the degree of oxidation of the fluorescent protein which is the basis for the assay indicating that melatonin prevents macromolecular damage that is a result of dopamine autooxidation. The authors surmised that this was due to the free radical scavenging capacity of melatonin and they suggested that the indole may have beneficial effects in reducing oxidative damage in the brain of PD patients.

While oxidative stress may be one feature that links many neurological deficits, it is also obvious that these diseases have extremely complex etiopathologies and it is unlikely that a single agent will totally combat their development. Moreover, there is an urgent need to understand the mechanisms underlying the degeneration of neurons.

Melatonin as a potential treatment to defer neurodegenerative diseases is of interest for several reasons: the endogenous production of this molecule falls with age coincident with the onset of many of the age-associated neurodegenerative conditions (Reiter et al., 1998); melatonin readily crosses the blood-brain barrier and after its exogenous administration it is found in high concentrations in the brain, sometimes exceeding those in the blood manifold (Reiter, 1995); melatonin is a ubiquitously acting free radical scavenger and antioxidant (Reiter et al., 1993) which in models of neurological diseases has proven effective in reducing oxidative damage and preserving neurological function (Reiter, 1998).

The importance of the study of melatonin as a promising molecule to understand better the pathogenesis of AD and PD is illustrated by the recent fact that soluble forms of full-length β-amyloid precursor protein (β-APP) of the Aβ-peptide were detected in secretory granules of chromaffin cells (Efthimiopoulos et al., 1996), where melatonin and dopamine are also synthesized (Kvetnoy, 1999).

Moreover, it was shown that stimulation of APP secretion was paralleled by a stimulation of secretion in catecholamines and chromogranin A, indicating that secretion of APP was mediated by chromaffin granules. Because, secretion of APP from primary chromaffin cells was time-dependent, we surmise that melatonin may have a direct effect on this process.

Serotonin (5-hydroxytriptamine; 5-HT). 5-HT neuron and neurotransmitter loss in normal ageing and neuropsychiatric diseases of late life may contribute to behavioral changes commonly observed in the elderly population (Meltzer et al., 1998). Extensive evidence implicates a deficit in serotoninergic neurotransmission in the development of major depression. The concentrations of 5-HT is reduced by 18% in the frontal cortex and by 21-37% in hippocampal cortex, hippocampus and striatum in AD (Reinikainen et al., 1990). It has been further suggested that the age-related changes in 5-HT neurons may predispose the elderly to depression. There is also increasing evidence that a disturbance in serotoninergic function may play a role in cognitive impairment in AD.

Catecholamines (CA). There are many data showing a significant loss of dopamine (DA) immunopositive neurons in the brain of PD patients (Li and Song , 2020). Also a reduction of DA concentrations (18-27% compared with normal level) have been noted in AD patients in the temporal cortex and hippocampus (Reinikainen et al., 1990).

Immunocytochemical techniques have been used to compare the proportion of neurons expressing CA in the different brain areas of neurologically normal elderly humans to that of age-matched AD patients (Yew et al., 1999). The CA cells in the frontal cortex of the AD patients were found to be significantly decreased; the CA are present in both cortical neurons and astrocytes. (Reinikainen et al., 1990) showed that in AD patients the concentration of noradrenaline was reduced (18-36% compared with normal patients) in frontal and temporal cortices, and in putamen.

Histamine. Histamine is known to be a neurotransmitter, but it has not been clearly implicated in major diseases. All histaminergic neurons reside in the posterior hypothalamus and innervate most brain areas, which is compatible with the

concept that histamine is involved in general central regulatory mechanisms. A sensitive high-performance liquid chromatographic fluorimetric method was used to measure histamine content in post mortem brain in AD patients and age-matched controls (Zlomuzica et al., 2016). At the same time the cellular storage sites and distribution of histaminergic fibers were examined with a specific immunohistochemical method.

The histamine content was significantly reduced in the hypothalamus (42% of control value), hippocampus (43%) and temporal cortex (53%) of AD brains. Histamine concentration in other cortical areas, putamen and substantia nigra were not significantly altered. Histamine-containing nerve fibers were found in the hippocampus, parahippocampal gyrus and subiculum of both AD brains and controls.

No histamine-containing mast cells were seen in these temporal structures. Histamine in the human temporal lobe is stored in nerve fibers originating from the posterior hypothalamus, and not in mast cells. A reduction in brain histamine levels may contribute to the cognitive decline in AD directly or through the cholinergic system. Thus, development of drugs that penetrate the blood-brain barrier and increase histaminergic activity may be beneficial in AD (Zlomuzica et al., 2016).

Somatostatin (ST). ST was originally isolated from hypothalamic extracts (Brazeau et al., 1973). It has subsequently been shown to be present in neurons and endocrine cells throughout the brain and gut (Figlewicz et al., 1987). Numerous central effects of ST have been described, although there appear to be some conflicts in the literature.

The consensus appears to be that main neurobiological effect of ST results in a generalized arousal, with concomitant enhancement of grooming and exploratory activities. Metabolically, it has been shown to inhibit the hyperglycemic response to a variety of stressors (Brown et al., 1979).

Disturbances in ST synthesis and secretion may play a role in the pathogenesis of various neurological diseases. Recent data suggest a disturbance of some brain ST neurons in AD, moreover, some endocrine activities known to be regulated by ST, such as growth hormone, thyroid-stimulating-hormone, somatomedins, as well as insulin and glucose, also seem to be affected in some patients (Reubi and Palacios, 1986). It is speculated that these changes are due to a global CNS and endocrine ST defect in AD and that the described endocrine imbalances may indirectly be responsible for at least part of the CNS pathology.

A deficiency in ST is the most consistently described neurochemical alteration in AD attributable to intrinsic cortical neurons (Beal et al., 1986). ST concentrations are depleted in cerebral cortex in both AD and in the dementia that accompanies PD (Beal, 1990). ST neurons in both illness are markedly dystrophic and may be reduced in number. Li et al. (1996) tried to verify if there is a difference in the number of ST neurons in the cortex between normal ageing versus AD patients and, secondly, if any of these neurons were dying via apoptosis.

In their specimens, immunohistochemistry revealed that there was no difference in the number of ST-containing neurons between the two study groups. Moreover, the bulk of the apoptotic cells that were idenified using the sensitive immunocytochemical TUNEL method, none contained ST (Li et al., 1996). It is concluded that while there is apoptotic cell death in normal ageing and AD, it does not seem to occur in neurons which contain ST in any significant amount.

A novel role for receptor-associated protein in ST modulation and it's implications for AD was shown recently. It is known that receptor-associated protein appears to play an important role in low-density lipoprotein receptor-related protein (LRRP) trafficking. Since ligands for the LRRP have been implicated in AD and normal functioning of this protein is indispensable for CNS development, deficient LRRP expression may result in CNS alterations (Solarski et al., 2018). In this study, receptor-associated protein-knockout mice were behaviorally tested and nervous system integrity was assessed via in situ hybridization and immunocytochemical/laser confocal microscopy methods.

In wild-type mice, the LRRP was found to be highly co-expressed with ST in hippocampal and neocortical inhibitory neurons. LRRP-knockout mice, however, showed a significant decrease in number of ST-expressing neurons in the CA1 region and ST expression within these neurons. The

decreased number of ST neurons significantly correlated with cognitive impairment observed in the receptor-associated protein in modulating the functioning of ST-producing neurons. Furthermore, this has implications for AD pathogenesis, in which altered regulation of both ST and the known LRRP ligands are a consistent finding.

Endogenous oppiates. Yew et al. (1999) obtained only minimal difference in the proportion of cortical neurons expressing leu-enkephalin between normal and AD patients.

Hypothalamic and pituitary peptides. The neuropathological hallmarks of neurodegenerative diseases are very prominent in the hippocampus (Kraskovskaya et al., 2017), a brain site that is pivotal for regulation of the synthesis of the hypothalamic and pituitary hormones. An alteration of neuroendocrine processes is supported by a significantly reduction of adrenocorticotropin hormone (ACTH) levels in cerebrospinal fluid in AD patients as compared with the controls (Suemaru et al., 1993).

Several studies indicate a reduction in corticotropin-releasing hormone (CRH) immunoreactivity in the cerebral cortex of AD patients (De Souza et al, 1986; Vandael and Gounko, 2019), particularly in temporal, frontal, and occipital areas. Nevertheless, these findings are not specific to AD. In fact, reduced levels of CRH in cerebrospinal fluid were also demonstrated in patients with vascular dementia (Suemaru et al., 1991), and reduced CRH immunoreactivity in cerebral cortex was found in PD.

An attenuated growth hormone-releasing hormone (GHRH)–induced growth hormone response specific to AD has been demonstrated (Chiso et al., 1993). Furthermore, a reduction in cerebrospinal fluid levels of antidiuretic hormone was observed not only in AD patients, but also in patients with frontal lobe dementia (Petrella et al., 2019). No alteration in the synthesis of thyrotropin-releasing hormone and prolactin was found in AD or PD (Dysken et al., 1990).

Substance P (SP). SP was first isolated and chemically characterized from hypothalamus. Immunohistochemical findings indicate that many nerve fibers from the amygdalo-fugal pathway, probably via the stria terminalis, contain SP, and enter the bed nucleus of the stria terminalis (Sakanaka et al., 1981) and lateral hypothalamus (Sakanaka et al., 1982).

Besides, the neurons with SP immunoreactivity have been observed in the arcuate nucleus, ventral and dorsal premammillary nucleus, dorsomedial nucleus, in the medial preoptic area, the periventricular nuclei of the dorsal tuberal region, and the lateral hypothalamus (Ljungdahl et al., 1978; Ronnekleiv et al., 1984).

These cells lack a projection to the median eminence but probably subserve important roles in integrating information from within the limbic system, including neuroendocrine regulation (Aronin et al., 1986). An important ultrastructural observation is that terminals containing SP form axodendritic synapses in the tuberoinfundibular region (Tsuruo et al., 1983).

SP immunoreactivity is present also in anterior pituitary and in median eminence (Sakanaka et al., 1981; Tsuruo et al., 1983). It seems to be possible that SP plays a role as a paracrine regulator of intrabrain hormonal status (Aronin et al., 1986). Immunocytochemical studies (Yew et al., 1999) have not documented a difference between the number and/or functional activity of cortical SP-immunoreactive neurons in healthy and AD patients in the same age.

Neurotensin (NT). Like SP, NT was also first isolated from hypothalamus. Numerous cells containing NT have been found in the paraventricular and periventricular cell groups and in the lateral hypothalamus. Both magno- and parvocellular neurons are labeled with NT in the paraventricular nucleus, which may indicate that NT-stained cells are components of the hypothalamic-anterior pituitary axis and the neurohypophyseal tract (Jennes et al., 1982).

Scattered NT-positive cells have been observed in other hypothalamic regions, with the exception of the supraoptic, suprachiasmatic, and ventromedial nuclei (Kahn et al., 1980). Dense fiber labeling is located in the paraventricular and periventricular zones and, importantly, in the median eminence (Kahn et al., 1982).

Some hypothalamic neurons that are positive for NT may also contain CA. These cells are distributed in the periventricular and arcuate regions (Liu et al., 2017). Like SP, NT immunoreactivity has been identified in anterior pituitary cells (Goedert et al., 1982). The role of NT in central neural functions is not defined; one idea is that

this peptide together with SP may regulate the content of other peptides in the brain (Aronin et al., 1986).

Cholecystokinin (CCK). The presence of cholecystokinin (CCK) in high concentrations in a number of brain areas, its colocalization with DA in some central neurons, the distinct behavioral effects it has, and the alterations in certain neurotransmitter systems that are seen following its peripheral or central administration, all implicate CCK as a neuromodulator or neurotransmitter (Figlewicz et al., 1987).

High concentrations of CCK in the CNS occur in the cortex, caudate nucleus and olfactory bulb (Goltermann, 1982). The effects of CCK in the CNS may involve its interaction with major neurotransmitter pathways. CCK injection into the lateral hypothalamus increases DA and noradrenaline bindings in the nucleus accumbens (Dumbrille-Ross and Seeman, 1984). There are some data indicating a decrease in the number of CCK immuno-positive neurons in cortex of post mortem AD brain (Plagman et al., 2019).

Bombesin (BOM). BOM immunoreactivity is localized in nerve cells in different areas of brain, but the largest amount of this peptide is present in the hypothalamus and brain tissue closely to fourth ventricle (Figlewicz et al., 1987) . Like many other gut peptides (e.g. CCK), found in brain, BOM has been shown to reduce meal size. Additionally, it has important neurobiological effects. Brown (1983) reported that BOM activates the adrenal medulla and results in markedly elevated plasma adrenaline levels, with secondary increases in plasma glucose and glucagon. Because noradrenaline is reduced in AD (Reinikainen et al., 1990), it is possible, that BOM may have therapeutical significance in maintaining normal levels of noradrenaline.

Neuropeptide Y (NPY). It is known, that NPY found in high concentrations in cerebral cortex and is contained in cortical neurons (Figlewicz et al., 1987). NPY-containing nerve fibers also innervate small blood vessels. NPY is colocalized with catecholamines in some areas of brain (Everitt et al., 1984), and chemical depletion of catecholamines results in depletion of NPY in some, but not all, neurons (Lundberg et al., 1985).

Beal et al. (1986) measured concentrations of this peptide in postmortem tissue from AD patients and controls using a sensitive and specific radioimmunoassay. High-performance liquid chromatography showed that more than 95% of immunoreactivity co-migrated with synthetic standards in both AD and control frontal cortex. Significant reductions in neuropeptide Y immunoreactivity were found in cortex, the hippocampus, and the locus ceruleus. The regions particularly affected included the temporal lobe, frontal lobe, and occipital cortex.

A reduction in immune function has been found in patients with a major depressive disorder and in persons undergoing severe life stress (Merrill and Jonakait, 1995). Irwin et al. (1991) investigated the association between NPY and natural killer (NK) cytotoxicity in AD depression. Circulating concentrations of NPY in plasma were inversely correlated with NK activity in AD patients. These findings suggest that the release of NPY may be associated with the modulation of NK cytotoxicity.

Insulin (INS). Earlier it was impossible to imagine the active production of insulin outside of pancreas, especially in CNS, but in the last few years new evidence has indicated that insulin and its receptors are present in the brain. Insulin was detected in brain by radioimmunoassay, biochemical and immunochemical methods (Baskin et al., 1987).

Immunohistochemical localization of insulin-containing neurons has been shown in many areas of brain, but the olfactory bulbs and hypothalamus consistently have the highest concentrations of insulin. Insulin provides for glucose utilization in brain tissue as well as is an important regulatory peptide in the CNS participated in many physiological processes (e.g. insulin inhibits firing of neurons in the hippocampus and hypothalamus).

Several lines of evidence indicate that insulin may influence synaptic activity. Insulin modulates monoamine uptake in cultured neuronal cells (Boyd et al., 1985) and stimulates synaptosomal uptake of neurotransmitter amino acids (Sun et al., 2018).

Insulin increases catecholamine turnover and release from brain cells and has also been shown to stimulate Na, K-ATPase activity in hippocam-

pus. Acquired disturbances of several aspects of cellular metabolism appear pathologically important in sporadic AD.

Among these, brain glucose utilization is reduced in the early stages of the disease and the regulatory enzymes important for glucose metabolism are reduced (Fine et al., 2017). In the brain, INS, insulin-like growth factors and their receptors regulate glucose metabolism and promote neuronal growth. INS and c-peptide concentration in the brain is decreased with ageing and AD (Fine et al., 2017).

Weak INS-immunoreactivity could be demonstrated histochemically in pyramidal neurons of controls, whereas in AD a stronger INS-immunoreactivity was found. Brain INS receptor densities in AD were decreased compared to middle-aged controls, but increased in comparison to age-matched controls. INS growth factor-I receptor densities were unchanged in ageng and in AD. Tyrosine kinase activity, a signal transduction mechanism common to both receptor systems, was reduced in AD in comparison to middle-aged and age-mathced control groups (Yew et al., 1999; Fine et al., 2017). These data are consistent with a neurotrophic role of INS in the human brain and a disturbance of INS signal transduction in AD brain and favor the hypothesis that INS dependent functions may be of pathogenetic relevance in sporadic AD.

Glucagon (GLU). In addition to being found in the pancreas and gut, GLU immunoreactivity has been demonstrated in brain, where the highest concentrations appear to be in the hypothalamus with intermediate amounts in the midbrain and low amounts in cortex (Figlewicz et al., 1987).

There are not many research data to establish a possible function of GLU in the CNS. GLU immunoreactivity is released from a synaptosomal preparation of thalamus, hypothalamus, and brain stem in response to K^+ (Tominaga et al., 1984).

These data support a potential role of CLU as neurotransmitter or neuromodulator, which could be involved in the cascade of molecular reactions that regulate intracellular Ca^{2+} concentration. Many data testify to dysfunction of intracellular Ca^{2+} homeostasis to result in neuronal loss (Choi, 1995; Verkhratsky et al., 1998).

Endothelins (ET). ET are a potent vasoactive peptides produced by endothelial cells that elecits prolonged constriction in most smooth muscle preparations and dilation in others (Rubanyi and Botelho, 1991). Of three isopeptides, ET-1 is the only form constitutively released and may modulate vascular tone via binding to one of several receptor subtypes in smooth muscle. ET-1 immunoreactivity in the AD brain was significantly increased in frontal and occipital cortex that those in the control brain and a significant correlation was found between frontal and temporal lobe of AD brains (Minami et al., 1996). These findings may explain the clinico-radiological results that the cerebral blood flow is decreased in AD patients, the mechanism of which is still unknown.

Chromogranin A (CGA). CGA belongs to a multifunctional peptide family widely distributed in secretory vesicles in neurons and neuroendocrine cells. Within the brain, CGA is localized in neurodegenerative areas associated with reactive microglia. CGA stimulated microglial cells to secrete heat-stable diffusible neurotoxic agents and also induced a marked accumulation of NO and tumor necrosis factor by microglia (Gasparini et al., 1998). It seems to be possible, that CGA represents an endogenous factor that triggers the microglial activity responsible for the pathogenesis of neuronal degeneration.

CONCLUSION: *Further investigations of brain hormones for improvement of diagnosis and therapy of neurodegenerative diseases*

In spite of many reports on the study of the behavior and role of different biologically active substances in the pathogenesis of mitochondrial diseases, most of them are devoting to concret one or two types of molecules. Thus, a complex studies, in which the behaviour of many molecules was studied at the same time and at the same patients are absent.

We guess, that it is now essential to identify not only those cytokines and other molecules above and their actions that are associated directly with physiological regulation and disease processes, but also their mechanisms of communications and joint actions. A clear understanding of these

processes, combined with development of methods to manipulate them, is likely to offer significant therapeutic potential in the successful treatment of mitochondrial diseases.

It is necessary to underline, that while the patient is alive, the distinction between different forms of dementia rests with clinical assessment. The diagnosis of a specific form of dementia as AD manifestation is confirmed only at autopsy. This circumstance dictates the necessity of the search of in lifetime markers for diagnosis and prognosis of AD and relative mitochondrial diseases.

It seems to be possible, because recently the results of some investigations (Gasparini et al., 1998; Miklossy et al., 1999) suggest that AD might be the disease not only the central nervous system, but might be a systemic disorder. If so, the use of human peripheral cells and tissue biopsies could provide a promising tool for lifetime diagnosis of AD and other neurodegenerative diseases.

We suppose, that the further investigations in this direction should include the following steps to understanding better the inter- and intracellular mechanisms of neurodegenerative pathology as well as for elaboration of a new promising life-time marker of these diseases. The main of these steps are following:

- ***to study the molecular bases of AD and PD associated with defects of the mitochondrial machinery of protein translocation*** (i.e. to complete the sequence of the hTom20 gene; to elucidate the chromosome location of the gene and processed pseudogenes; to identify polymorphism of the hTom20 gene in general population as well as in patients with AD and PD; to identify new subunits of the translocase of the mitochondrial outer membrane (Tom complex), and to characterize their respective genes).

- ***immunocytochemical mapping and image analysis of microscopic manifestations of AD and PD*** (to identify localization of the most key molecules which are involved in pathogenesis of neurodegenerative diseases in human brain; to study in detail a functional morphology of cells and tissue structures imunocytochemically positive to molecules above and to compare them to the pathological lesions on light and electron-microscopical levels in post-mortem brain in AD and PD patients).

- ***development of a new methods for lifetime diagnosis and treatment of AD and PD*** (taking into account an important role of MT as scavenger of free radicals, it seems to be very promising to clarify the role of MT in intracellular mechanisms of neurodegenerative diseases and to elaborate it as a new in lifetime marker for diagnostics of AD and PD. To achieve this aim the objectives below should be solved: 1) to identify the key molecules of these disorders (i.e. β-APP, tau-protein, ubiquitin, dopamine, NO-synthase) and MT in human blood lymphocytes from healthy volunteers and patients with AD and PD, and thus to show that blood lymphocytes could be used as a suitable object for lifetime diagnosis of AD and PD; 2) to carry out the quantitative immunocytochemical analysis of concentration of β-APP, tau-protein, ubiquitin, dopamine, NO-synthase and MT in the lymphocytes from AD and PD patients; 3) to determine the excretion in urine of the products of MT interactions with free radicals: cyclic 3-hydroxymelatonin (3-HMT), N^1-acetyl-N^2-formyl-5-methoxykynuramine (AMFK), N^1-acetyl-5-methoxykynuramine (AMK) (Lee et al., 2016) as well as 6-sulfatoxymelatonin (aMT6s) in AD and PD patients; 4) to identify of polymorphisms and mutations in Tom20 gene in humal blood lymphocytes of general population and patients with AD and PD; 5) to study correllations between expression of Tom20 gene, β-APP, tau-protein, ubiquitin, dopamine, NO-synthase and MT in human lymphocytes of healthy people as well as of AD and PD patients and compare them with excretion indices of the same groups; 6) on the basis of data of the research above to clarify the biological role of MT in intracellular mechanisms of neurodegenerative diseases and to elaborate aMT6s as a new non-invasive marker for lifetime diagnosis, definition of prognosis and efficiency of specific therapy in individual AD and PD patients).

Thus, there is no doubt, that DNIES and its hormones being a multifunctional biologically active molecules and located everywhere in the organism, including brain play an important role in

pathogenesis of AD, PD, and other neurodegenerative and mitochondrial diseases. The further investigations in this field of study it seems to be very effective as well as for elucidation of molecular and cellular bases of pathological mechanisms of neuronal degeneration and moreover for elaboration of optimal methods of diagnosis and therapy of many diseases.

ACKNOWLEDGEMENTS

This research was supported by RFFI (Russia) grant 18-54-06012 Az_a and SDF (Azerbaijan) grants EIF-BGM-4-RFTF-1/2017-21/19/3 and EIF-KETPL-2-2015-1(25)-56/36/3-M-54.

REFERENCES

Acuna-Castroviejo D., Coto-Montes A., Monti M.G. et al. (1997) Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life Sci.*, **60:** 23-29.

Alves-Rodrigues A., Gregori L., Figueiredo-Pereira M.E. (1998) Ubiquitin, cellular inclusions and their role in neurodegeneration. *Trends Neurosci.*, **21:** 516-520.

Ames B.N., Shigenaga M.K., Hagen T.M. (1993) Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA*, **90:** 7915-7922.

Aronin N., Coslovsky R., Leeman S.E. (1986) Substance P and neurotensin**:** their role in the regulation of anterior pituitary function. *Annu. Rev. Physio.l*, **48:** 537-549.

Asensio V.C., Campbell I.L. (1999) Chemokines in the CNS**:** plurifunctional mediators in diverse states. *Trends Neurosc.i*, **22:** 504-512.

Auld D.S., Kar S., Quirion R. (1998) β-Amyloid peptides as direct cholinergic neuromodulators**:** a missing link? *Trends Neurosci.*, **21:** 43-49.

Barlow-Walden L.R., Reiter R.J., Abe M. et al. (1995) Melatonin stimulates brain glutathione peroxidase activity. *Neurochem. Int.*, **26:** 497-502.

Baskin D.G., Figlewicz D.P., Woods S.C. et al. (1987) Insulin in the brain. *Annu. Rev. Physiol.*, **49:** 335-347.

Beal M.F., Benoit R., Mazurek M.F. et al. (1986) Somatostatin-28 (1-12)-like immunoreactivity is reduced in Alzheimer's disease cerebral cortex. *Brain Res*, **368:** 380-383.

Beal M.F. (1990) Somatostatin in neurodegenerative illnesses. *Metabolism*, **39 (9, Suppl 2):** 116-119.

Blass J.P., Baker A.C., Ko L. et al. (1991) Expression of "Alzheimer antigens" in cultured skin fibroblasts. *Arch. Neurol.*, **48:** 709-717.

Bolanos J.P., Almeida A., Stewart V. et al. (1997) Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J. Neurochem.*, **68:** 2227-2240.

Borjigin J., Li X., Snyder S.H. (1999) The pineal gland and melatonin: molecular and phar-macologic regulation. *Annu. Rev. Pharmacol. Toxicol*., **39:** 53-65.

Boyd F.T., Clarke D.W., Muther T.F., Raizada M.K. (1985) Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J. Biol. Chem.*, **260:** 15880-15884.

Brazeau P., Vale W., Burgus R. et al. (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science*, **179:** 77-79.

Brown M., Rivier J., Vale W. (1979) Somatostatin: central nervous system actions on glucoregulation. *Endocrinology*, **104:** 1709-1715.

Brown M.R. (1983) Central nervous system sites of action of bombesis and somatostatin to influence plasma epinephrine levels. *Brain Res.*, **276:** 253-257.

Calhoun M.E., Wiederhold K-H., Abramowski D. et al. (1998) Neuron loss in APP transgenic mice. *Nature*, **395:** 755-756.

Chiso E., Nicolosi M., Arvat E. et al. (1993) Growth hormone secretion in Alzheimer's disease: studies with growth hormone releasing hormone alone and combined with pyridostigmine or arginine. *Dementia*, **4:** 315-320.

Choi D.W. (1995) Calcium: still center-stage in hypoxic-ishemic neuronal death. *Trends Neurosci.*, **18:** 58-60.

Ciesielski-Treska J., Ulrich G., Taupenot L. et al. (1998) Chromogranin A induces a neurotoxic phenotype in brain microglial cells. *J. Biol. Chem*., **273:** 14339-14346.

Clark E.A., Brugge J.S. (1995) Integrins and signal transduction pathways: the road taken. *Science,* **268:** 233-238.

Clayton D.F., George J.M. (1998) The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.*, **21:** 249-254.

Congdon E.E., Sigurdsson E.M. (2018) Tau-targeting therapies for Alzheimer disease. *Nat Rev Neurol.*, **14(7):** 399-415.

Coyle J.T., Puttfarcken P. (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science,* **262:** 689-695.

Cuzzocrea S., Tan D-X., Costantino G. et al. (1999) The protective role of endogenous melatonin in carrageenan-induced pleurisy in the rat. *FASEB J.*, **13:** 1930-1938.

Davies A.M. (1995) The bcl-2 family of proteins, and the regulation of neuronal survival. *Trends Neurosci.*, **18:** 355-358.

Davis R.E., Miller S., Herrnstadt C. et al. (1997) Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA*, **94:** 4526-4531.

De Souza E.B., Whitehouse P.J., Kuhar M.J. et al. (1986) Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature*, **319:** 593-595.

Dehghani Z., Meratan A.A., Saboury A.A., Nemat-Gorgani M. (2020) α-Synuclein fibrillation products trigger the release of hexokinase I from mitochondria: Protection by curcumin, and possible role in pathogenesis of Parkinson's disease. *Biochim. Biophys. Acta Biomembr.*, **1862(6):** 183251.

Devine M.J., Kittler J.T. (2018) Mitochondria at the neuronal presynapse in health and disease. *Nat. Rev. Neurosci.*, **19(2):** 63-80.

Dickson D.W. (1997) The pathogenesis of senile plaques. *J. Neuropathol. Exp. Neurol*., **56:** 321-339.

Dines K.C., Powell H.C. (1997) Mast cell interactions with the nervous system: relationship to mechanisms of disease. *J. Neuropathol. Exper. Neurol.*, **56:** 627-640.

Dumbrille-Ross A., Seeman P. (1984) Dopamine receptor elevation by cholecystokinin. *Peptides*, **5:** 1207-1212.

Dysken M.W., Falk A., Pew B. et al. (1990) Gender differences in TRH-stimulated TSH and prolactin in primary degenerative dementia and elderly controls. *Biol. Psychiatry*, **28:** 144-150.

Efthimiopoulos S., Vassilacopoulou D., Ripellino J.A. et al. (1996) Cholinergic agonists stimulate secretion of soluble full-length amyloid precursor protein in neuroendocrine cells. *Proc. Natl. Acad. Sci. USA*, **93:** 8046-8050.

Everitt B.J., Hokfelt T., Terenius L. et al. (1984) Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. *Neuroscience*, **11:** 443-462.

Fadeel B., Zhivotovsky B., Orrenius S. (1999) All along the watchtower: on the regulation of apoptosis regulators. *FASEB J.*, **13:** 1647-1657.

Fahn S., Cohen G. (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann. Neurol.*, **32:** 804-812.

Figlewicz D.P., Lacour F., Sipols A. (1987) Gastroenteropancreatic peptides and the central nervous system. *Annu. Rev. Physiol.*, **49:** 383-395.

Fine J.M., Forsberg A.C., Stroebel B.M. et al. (2017) Intranasal deferoxamine affects memory loss, oxidation, and the insulin pathway in the streptozotocin rat model of Alzheimer's disease. *J. Neurol. Sci*., **380:** 164-171.

Gale L.M., McColl S.R. (1999) Chemokines: extracellular messengers for all occasions. *BioEssaya,* **21:** 17-28.

Gao Y., Tan L., Yu J.T., Tan L. (2018) Tau in Alzheimer's Disease: Mechanisms and Therapeutic Strategies. *Curr. Alzheimer Res.*, **15(3):** 283-300.

Gasparini L., Racchi M., Binetti G. et al. (1998) Peripheral markers in testing pathophysiological hypotheses and diagnosing Alzheimer's disease. *FASEB J.*, **12:** 17-34.

Goedert M., Lightman S.L., Nagy J.I. et al. (1982) Neurotensin in the rat anterior pituitary gland. *Nature*, **298:** 163-165.

Goltermann N.R. (1982) In vivo biosynthesis of cholecystokinin in hog cerebral cortex. *Peptides*, **3:** 101-104.

Green D.R. (1998) Apoptotic pathways: the roads to ruin. *Cell*, **94:** 695-698.

Griffin S.W.T, Sheng J.G., Roberts G.W., Mrak R.E. (1995) Interleukin-1 expression in different plaque types in Alzheimer's disease: significance in plaque evolution. *J. Neuropathol. Exp. Neurol*., **54:** 276-281.

Grota L.J., Brown G.M. (1974) Antibodies to indolealkylamines: serotonin and melatonin. *Can. J. Biochem.*, **52:** 196-203.

Hachiya N., Alam R., Sakasegawa N. et al. (1993) A mitochondrial import factor purified from rat liver cytosol is an ATP-dependent conformational modulator for precursor proteins. *EMBO J.*, **12:** 1579-1586.

Hernandez J.M., Giner P., Hernandez-Yago J. (1999b) Gene structure of the human mitochondrial outer membrane receptor Tom20 and evolutionary study of its family of processed pseudogenes. *Gene*, **239:** 283-291.

Hernandez J.M., Hernandez C.S., Giner C.P. et al. (1999a) Identification of Ψ3Tom20, a novel processed pseudogene of the human Tom20 gene, and complete characterization of Ψ1Tom20 and Ψ2Tom20. *Mol. Gen. Genet.,* **262:** 207-211.

Hockenbery D, Nunez G, Milliman C et al. (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature*, **348:** 334-336.

Hockenbery D.M., Oltvai Z.N., Yin X-M. et al. (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell*, **75:** 241-251.

Iadanza M.G., Jackson M.P., Hewitt E.W. et al. (2018) A new era for understanding amyloid structures and disease. *Nat. Rev. Mol. Cell. Biol.*, **19(12):**755-773.

Irwin M., Brown M., Patterson T. et al. (1991) Neuropeptide Y and natural killer cell activity: findings in depression and Alzheimer caregiver stress. *FASEB J.*, **5:** 3100-3107.

Ishii M., Hiller A.J., Pham L. et al. (2019) - amyloid-beta modulates low-threshold activated voltage-gated L-type calcium channels of arcuate neuropeptide y neurons leading to calcium dysregulation and hypothalamic dysfunction. *J. Neurosci.*, **39(44):** 8816-8825.

Iwai A., Masliah E., Yoshimoto M. et al. (1995) The precursor protein of non-Aβ component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron*, **14:** 467-475.

Jacobson M.D., Burne J.F., King M.P. et al. (1993) Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature*, **361:** 365-369.

Jennes L., Stumpf W.E., Kalivas P.W. (1982) Neurotensis: topographical distribution in rat brain by immunohistochemistry. *J. Comp. Neurol.*, **210:** 211-224.

Kahn D., Abrams G.M., Zimmerman E.A. et al. (1980) Neurotensin neurons in the rat hypothalamus: an immunocytochemical study. *Endocrinology*, **107:** 47-54.

Kahn D., Hou-You A., Zimmerman E.A. (1982) Localization of neurotensin in the hypothalamus. *Ann. NY Acad. Sci.*, **400:** 117-131.

Kang P.J., Ostermann J., Shilling J. et al. (1990) Hsp70 in the mitochondrial matrix is required for translocation and folding of precursor proteins. *Nature*, **348:** 137-143.

Koenig H.L., Schumacher M., Ferzaz B. et al. (1995) Progesterone synthesis and myelin formation by Schwann Cells. *Science*, **268:** 1500-1503.

Kraskovskaya NA, Kukanova EO, Linkova NS et al. (2017) Tripeptides Restore the Number of Neuronal Spines Under Conditions of *In Vitro* Modeled Alzheimer's Disease. *Bulletin of Experimental Biology and Medicine*, **163(4):** 550-553.

Kreutzberg G.W. (1996) Microglia: a sensor for pathological events in CNS. *Thends Neurosci*., **19:** 312-318.

Kroemer G., Dallaporta B., Resche-Rigon M. (1998) The mitochondrial death/life regulator in apoptosis and necrosis. *Annu. Rev. Physiol*., **60:** 619-642.

Kunkl M., Frascolla S., Amormino C. et al. (2020) T Helper Cells: The Modulators of Inflammation in Multiple Sclerosis. *Cells*, **9(2): pii:** E482.

Kvetnoy I.M., Sandvik A.K., Waldum H.L. (1997) The diffuse neuroendocrine system and extrapineal melatonin. *J. Mol. Endocrinol.,* **18:** 1-3.

Kvetnoy I.M., Yuzhakov V.V., Raikhlin N.T. (1997) APUD cells: modern strategy of morphofunctional analysis. *Microsc. Anal.*, **48:** 25-27.

Kvetnoy I.M. (1999) Extrapineal melatonin: location and role within diffuse neuroendocrine system. *Histoch. J.*, **31:** 1-12.

Larrson L-I. (1980) On the possible existence of multiple endocrine, paracrine and neurocrine messengers in secretory system. *Invest Cell Pathol*., **3:** 73-85.

Lee K., Zawadzka A., Czarnocki Z. et al. (2016) Molecular cloning of melatonin 3-hydroxylase and its production of cyclic 3-hydroxymelatonin in rice (Oryza sativa). *J. Pineal. Res.*, **61(4):** 470-478.

Lee VM-Y., Trojanowski Q. (1999) Neurodegenerative tauopathies: human disease and transgenic mouse models. *Neuron*, **24:** 507-510.

Li P., Song C. (2020) Potential treatment of Parkinson's disease with omega-3 polyunsatu-rated fatty acids. *Nutr. Neurosci*., 1-12. doi: 10.1080/1028415X.2020.1735143

Li W.P., Lai H.W., Cheng S.Y., Yew D.T. (1996) Somatostatin-positive neurons in the different parts of the brain in normal aging and Alzheimer's disease. *Biol. Signals*, **5:** 343-348.

Lithgow T., Ryan M., Anderson R.L. et al. (1993) A constitutive form of heat-shock protein 70 is located in the outer membranes of mitochondria from rat liver. *FEBS Lett.*, **332:** 277-281.

Liu Q., Hazan A., Grinman E., Angulo J.A. (2017) Pharmacological activation of the neurotensin receptor 1 abrogates the methamphetamine-induced striatal apoptosis in the mouse brain. *Brain Res.*, **1659:** 148-155.

Ljungdahl A., Hokfelt T., Nillson G. (1978) Distribution of substance P-like immunoreactivity in the central nervous system of the rat. I. Cell bodies and nerve terminals. *Neuroscience*, **3:** 861-943.

Luft R. (1994) The development of mitochondrial medicine. *Proc. Natl. Acad. Sci. USA*, **91:** 8731-8738.

Lundberg J.M., Saria A., Franco-Cereceda A. et al. (1985) Differential effects of reserpine and 6-hydroxydopamine on neuropeptide Y (NPY) and noradrenaline in peripheral neurons. *Arch. Pharmacol.*, **328:** 331-340.

Lynn W.S., Wong P.K.Y. (1995) Neuroimmunodegeneration: do neurons and T cells use common pathways for cell death. *FASEB J.*, **9:** 1147-1156.

Martinus R.D., Ryan M.T., Naylor D.J. et al. (1995) Role of chaperones in the biogenesis and maintenance of the mitochondrion. *FASEB J.*, **9:** 371-378.

Mayo J.C., Sainz R.M., Uria H. et al. (1998) Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells**:** implications for Parkinson's disease. *J. Pineal. Res.*, **24:** 179-192.

Meltzer C.C., Smith G., DeKosky S.T. et al. (1998) Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. *Neuropsychopharmacology*, **18:** 407-430.

Mennicken F., Maki R., de Souza E.B., Quirion R. (1999) Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning. *Trends Pharmacol. Sci.*, **20:** 73-78.

Merrill J.E., Jonakait G.M. (1995) Interactions of the nervous and immune systems in development, normal brain homeostasis, and disease. *FASEB J.*, **9:** 611-618.

Mihara K., Omura T. (1996) Cytoplasmic chaperones in precursor targeting to mitochondria: the role of MSF and hsp70. *Trends Cell Biolm.*, **6:** 104-108.

Miklossy J., Taddei K., Martins R. et al. (1999) Alzheimer disease: curly fibers and tangles in organs other than brain. *J. Neuropathol. Exp. Neurol.*, **58:** 803-814.

Minami M., Kimura M., Iwamoto N., Arai H. (1996) Endothelin-1-like immunoreactivity in cerebral cortex of Alzheimer-type dementia. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **19:** 509-513.

Molnar M.J., Kovacs G.G. (2017) Mitochondrial diseases. *Handb. Clin. Neurol.*, **145:** 147-155.

Monaghan P, Robertson D, Amos TAS et al. (1992) Ultrastructural localization of bcl-2 protein. *J. Histochem. Cytochem.*, **40:** 1819-1825.

Morganti-Kossmann M.C., Kossmann T., Wahl S.M. (1992) Cytokines and neuropathology. *Trends Pharmacol. Sci.*, **13:** 286-291.

Mrak R.E., Griffin S.T., Graham D.I. (1997) Aging-associated changes in human brain. *J. Neuropathol. Exp. Neurol*., **56:** 1269-1275.

Nathan C. (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J.*, **6:** 3051-3064.

Neumann H., Wekerle H. (1998) Neuronal control of the immune response in the central nervous system: linking brain immunity to neurodegeneration. *J. Neuropathol. Exp. Neurol.*, **57:** 1-9.

Pallares-Trujillo J., Lopez-Soriano F.J., Argiles J.M. (1998) The involvement of the ubiquitin system in Alzheimer's disease (review). *Int. J. Mol. Med.*, **2:** 3-15.

Pang S.F. Dubocovich M.L., Brown G.M. (1993) Melatonin receptors in peripheral tissues: a new area of melatonin research. *Biol. Signals*, **2:** 177-180.

Papadopoulos V., Guarneri P., Krueger K.E. et al. (1992) Pregnenolone biosynthesis in C6-2B glioma cell mitochondria: regulation by a mitochondrial diazepam binding inhibitor receptor. *Proc. Natl. Acad. Sci. USA*, **89:** 5113-5117.

Paul S.M., Purdy R.H. (1992) Neuroactive steroids. *FASEB J.*, **6:** 2311-2322.

Pearse A.G.E. (1968) Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (the APUD series) and their relevance to thyroid and ultimobranchial C-cells and calcitonin. *Proc. Roy. Soc. B.*, **170:** 71-80.

Pearse A.G.E. (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. *J. Histochem. Cytochem.*, **17:** 303-313.

Petrella C., Di Certo M.G., Barbato C. et al. (2019) Neuropeptides in Alzheimer's Disease: An Update. *Curr. Alzheimer Res.*, **16(6):** 544-558.

Phu L., Rose C.M., Tea J.S. et al. (2020) Dynamic Regulation of Mitochondrial Import by the Ubiquitin System. *Mol. Cell*., **77(5):** 1107-1123.e10.

Plagman A, Hoscheidt S, McLimans KE et al. (2019) Cholecystokinin and Alzheimer's disease: a biomarker of metabolic function, neural integrity, and cognitive performance. *Neurobiol Aging.*, **76:** 201-207.

Polak J.M., Bloom S.R. (1986) Immunocytochemistry of the diffuse neuroendocrine system. In: J.M.Polak, S.Van Noorden (eds.) *Immunocytochemistry: modern methods and applications*. John Wright & Sons: 328-348.

Price D.L. (1999) New order from neurological disorders. *Nature*. **399 (Suppl):** A3-A5.

Raff M.C., Barres B.A., Burne J.F. et al. (1993) Programmed cell death and the control of cell survival: lessons from the nervous system. *Science*, **262:** 695-700.

Raikhlin N.T., Kvetnoy I.M. (1994) The APUD system (diffuse endocrine system) in normall and pathological states. *Physiol. Gen. Biol. Rev.*, **8(4):** 1-44.

Ramage L., Junne T., Hahne K. et al. (1993) Functional cooperation of mitochondrial protein import receptors in yeast. *EMBO J.*, **12:** 4115-4123.

Reinikainen K.J., Soininen H., Riekkinen P.J. (1990) Neurotransmitter changes in Alzheimer's disease**:** implications to diagnostics and therapy. *J. Neurosci. Res.*, **27:** 576-586.

Reiter R.J., Guerrero J.M., Garcia J.J., Acuna-Castroviejo D. (1998) Reactive oxygen intermediates, molecular damage, and aging. Relation to melatonin. *Ann. NY Acad. Sci.*, **854:** 410-424.

Reiter R.J., Poeggeler B,. Tan D-X. et al. (1993) Antioxidant capacity of melatonin: a novel function not requiring a receptor. *Neuroendocr. Lett.*, **15:** 103-116.

Reiter R.J. (1973) Comparative physiology: pineal gland. *Annu Rev Physiol*, **35:** 305-328.

Reiter R.J. (1992) The ageing pineal gland and its physiological consequences. *BioEssays.*, **14:** 169-175.

Reiter R.J. (1995) Oxidative processes and antioxidative defense mechanisms in the aging brain. *FASEB J.*, **9:** 526-533.

Reiter RJ.. (1997) Aging and oxygen toxicity: relation to changes in melatonin. *Age*, **20:** 201-213.

Reiter R.J. (1998) Oxidative damage in the central nervous system: protection by melatonin. *Prog. Neurobiol.*, **56:** 359-384.

Reubi J.C., Palacios J. (1986) Somatostatin and Alzheimer's disease: a hypothesis. *J. Neurol.*, **233:** 370-372.

Ronnekleiv O.K., Kelly M.J., Eskay R.L. (1984) Distribution of immunoreactive substance P neurons in the hypothalamus and pituitary of the rhesus monkey. *J. Comp. Neurol.*, **224:** 51-59.

Rothwell N.J., Hopkins S.J. (1995) Cytokines and the nervous system. II. Actions and mechanisms of action. *Trends Neurosci.*, **18:** 130-136.

Rubanyi G.M., Botelho H.P. (1991) Endothelins. *FASEB J.*, **5:** 2713-2720.

Sakanaka M., Shiosaka S., Takatsuki K. et al. (1981) Experimental immunohistochemical studies on the amygdalofugal peptidergic (substance P and somatostatin) fibers in the stria terminalis of the rat. *Brain Res.*, **221:** 231-242.

Sakanaka M., Shiosaka S., Takatsuki K. et al. (1982) Origins of substance P-containing fibers in the lateral septal area of young rats: immunohistochemical analysis of experimental manipulations. *J. Comp. Neurol.*, **212:** 268-277.

Sastry P.S., Rao K.S. (2000) Apoptosis and the nervous system. *J. Neurochem.*, **74:** 1-20.

Schapira A.H.V. (1993) Mitochondrial cytopathies. *Curr. Opin. Neurobiol*., **3:** 760-767.

Selkoe D.J. (1998) The cell biology of β-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Bio.l*, **8:** 447-453.

Shigenaga M.K., Hagen T.M., Ames B.N. (1994) Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. USA*, **91:** 10771-10778.

Silver R., Silverman A-J., Vitkovic L., Lederhendler I.I. (1996) Mast cells in the brain: evidence and functional significance. *Trends Neurosci.*, **19:** 25-31.

Smith M.A., Sayre L.M., Monnier V.M., Perry G. (1995) Radical AGEing in Alzheimer's disease. *Trends Neurosci.*, **18:** 172-176.

Solarski M., Wang H., Wille H., Schmitt-Ulms G. (2018) Somatostatin in Alzheimer's disease: A new Role for an Old Player. *Prion.*, **12(1):** 1-8.

Spillantini M.G., Goedert M. (1998) Tau protein pathology in neurodegenerative diseases. *Trends Neurosci.*, **21:** 428-433.

Spillantini M.G., Schmidt M.L., Lee VM-Y. et al. (1997) α-Synuclein in Lewy bodies. *Nature*, **388:** 839-840.

Suemaru S., Hashimoto K., Ogasa T. et al. (1991) Cerebrospinal fluid and plasma corticotropin-releasing hormone in senile dementia. *Life Sci.*, **48:** 1871-1879.

Suemaru S., Suemaru K., Hashimoto K. et al. (1993) Cerebrospinal fluid corticotropin-releasing hormone and ACTH, and peripherally circulating choline-containing phospholipid in senile dementia. *Life Sci.*, **53:** 697-706.

Sun P., Ortega G., Tan Y. et al. (2018) Streptozotocin impairs proliferation and differentiation of adult hippocampal neural stem cells *in vitro*-correlation with alterations in the expression of proteins associated with the insulin system. *Front. Aging Neurosci*., **10:**145.

Sundler F., Bottcher G., Ekblad E., Hakanson R. (1989) The neuroendocrine system of the gut. *Acta Oncol.*, **283:** 303-314.

Tan D.X., Manchester L.C., Reiter R.J. et al. (1999) Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochim. Biophys. Acta*, **1472:** 206-214.

Tominaga M., Kaneda H., Marubashi S. et al. (1984) Synaptosomal localization and release of glucagon-like materials in the rat brain. *Brain Res. Bull.*, **12:** 373-375.

Tsuruo Y., Kawano H., Nishimaya T. et al. (1983) Substance P-like immunoreactive neurons in the tuberoinfundibular area of rat hypothalamus. Light and electron microscopy. *Brain Res.*, **289:** 1-9.

Tsutsui K., Ukena K. (1999) Neurosteroids in the cerebellar Purkinje neuron and their actions (review). *Int. J. Mol. Med.*, **4:** 49-56.

Ueda K., Fukushima H., Maslian E. et al. (1993) Molecular cloning of a novel component of amyloid in Alzheimer's disease. *Proc. Natl. Acad. Sci. USA*, **90:** 11282-11286.

Vandael D., Gounko N.V. (2019) Corticotropin releasing factor-binding protein (CRF-BP) as a potential new therapeutic target in Alzheimer's disease and stress disorders. *Transl. Psychiatry*, **9(1):** 272.

Verkhratsky A., Toescu E.C. (1998) Calcium and neuronal ageing. *Trends Neurosci.*, **21:** 2-7.

Yew D.T., Li W.P., Webb S.E. et al. (1999) Neurotransmitters, peptides, and neural cell adhesion molecules in the cortices of normal elderly humans and Alzhemer patients: a comparison. *Exp. Gerontol.*, **34:** 117-133.

Zlomuzica A., Dere D., Binder S. et al. (2016) Neuronal histamine and cognitive symptoms in Alzheimer's disease. *Neuropharmacology*, **106:** 135-45.

Neurodegenerasiya mitoxondrial patologiya kimi: Siqnal mexanizmləri və *life-time* diaq-nostika və *target* terapiyasina yeni yanaşmalar

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Mitoxondrilərin struktur və funksional dəyişikliklərin "mitoxondrial xəstəliklər" adlandırılan çoxsaylı kliniki pozulmaların yaranmasının səbəbi olması məlumdur. Hal-hazırda mitoxondrial zülalların nəqlində bir çox amillərin, o cümlədən sitokinlərin, şaperonların, hemokinlərin, neyrosteroidlərin, ubikvitin və digərlərinin iştirakı şübhə doğurmur. Eyni zamanda mitoxondrial xəstəliklərin endogen mexanizmlərində biogen aminlərin və peptid hormonlarının (müxtəlif orqanlarda olan diffuz neyroendokrin sisteminin hüceyrələrində yaranırlar) iştirakı və rolu hələ də məlum deyil. Biogen aminlərin və peptid hormonlarının geniş spektrə malik bioloji effektlərini və xüsusilə hüceyrədaxili kommunikasiyada onların tənzimləyici rolunu nəzərə alaraq, bu molekulların mitoxondrial pozulmaların patogenezində mümkün iştirakının təhlil edilməsini, eləcə də neurodegenerativ xəstəliklərdə *lifetime* diaqnostika üçün yeni markerlərin işlənib hazırlanması, spesifik terapiyanın proqnozunun və effektivliyinin müəyyən edilməsi üçün yeni yanaşmaların işlənməsini mühüm tədqiqat hədəfləri kimi təqdim edirik.

Açar sözlər: *Mitoxondrial xəstəliklər, biogen aminlər, peptid hormonları, diffuz neuroimmunoendokrin sistem, life-time diaqnostikası, neyrodegenerasiya*

Нейродегенерация как митохондриальная патология: Сигнальные механизмы и новые пути для *прижизненной* диагностики и *таргетной* терапии

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Структурные и функциональные изменения митохондрий ответственны за широкий спектр клинических нарушений, которые называют “митохондриальными заболеваниями”. В настоящее время, очевидно, что в транспорте митохондриальных белков, таких как цитокины, шапероны, хемокины, нейростероиды, убиквитин и многие другие, участвуют многие факторы. В то же время участие и роль биогенных аминов и пептидных гормонов (которые продуцируются клетками диффузной нейроэндокринной системы, находящимися в различных органах) в эндогенных механизмах возникновения митохондриальных заболеваний до сих пор неизвестны. Учитывая широкий спектр биологических эффектов биогенных аминов и пептидных гормонов, и, особенно, их регуляторную роль во внутриклеточной коммуникации, представляется важным проанализировать возможное участие этих молекул в патогенезе митохондриальных нарушений, а также искать инновативные пути разработки новых маркеров для прижизненной диагностики, определения прогноза и эффективности специфической терапии при нейродегенеративных заболеваниях.

Ключевые слова: *Митохондриальные заболевания, биогенные амины, пептидные гормоны, диффузная нейроиммуноэндокринная система, life-time диагностика, нейродегенерация*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 89-96 (2020)

Bioeffects of electromagnetic irradiation on blood of rats

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Accepted for publication: 12 March 2020

Electromagnetic radiation (EMR) generated by various devices (radars, mobile phones, medical equipment) causes biochemical changes in the blood of humans and animals. Study of the effects of 460 MHz EMR on the blood levels of lipid peroxidation, total antioxidant activity (TAA) and reduced glutathione in the rats. Rats weighing 250−300g were subjected to the whole body exposure with 460 MHz EMR at the power density of 30 μW/cm^2 for 4 weeks, 6 days/week, 20 min/day. For this intensity of radiation, value of specific absorption rate (SAR) was estimated as 15 mW/kg. A dramatic increase in plasma MDA concentration was observed in the 1st week of exposure, which gradually decreased to a level slightly higher than that of control for other exposure periods. Erythrocyte MDA concentration was higher than that for the control animals for the 2nd and 4th weeks. TAA changes in the plasma and erythrocytes were little and statistically insignificant. Reduced glutathione concentration was found to decrease significantly by the end of the 4th week of exposure ($p<0.01$). The results indicate that free radical processes and antioxidant protection in the cells are involved in the mechanism of bioeffects of electromagnetic radiation in microwaves frequency range.

Keywords: *Electromagnetic radiation, plasma, erythrocytes, lipid peroxidation, antioxidant activity, reduced glutathione*

INTRODUCTION

In the modern world, the density of electromagnetic radiation (EMR) in the environment is growing steadily. This is due to the constant growth in the use of technical means that generate electromagnetic radiation in a variety of areas of human activity. Today, it is quite impossible to imagine the life of every person without devices and equipment of communication by the use of computer technology, EMR sources are intensively applied in medicine, military and household appliances, as well. The emergence of mobile communications has brought the problem of electromagnetic pollution of the environment to new aspect, i.e., now almost every person carries “their own individual electromagnetic environment”. Therefore, the study of the influence of such close to the human body sources is of great interest for the researchers, involved in the identification of biological effects of electromagnetic radiation and elucidation of their molecular mechanisms.

One of important areas of researches in electromagnetic biology is the study of the effect on the body of microwave electromagnetic irradiation. In particular, the sources of microwave radiation are mobile phones and their base stations, physiotherapy units and radars. They radiate in the frequency range corresponding to decimeter, centimeter and millimeter wavelengths. There are a lot of scientific works that are devoted to the study of changes in individual organs and tissues exposed to microwave radiation. The general focus of these studies is to identify the effect of low - intensity microwave irradiation on oxidative metabolism in different organs and tissues (Kivrak et al., 2017). Such processes as lipid peroxidation, antioxidant protective reactions in the brain, heart, liver, kidney and blood cells of the experimental animals, exposed to microwaves, were studied (Oktem et al., 2005; Elhag et al., 2007; Moussa, 2009; Megha et al., 2012; Alghamdi et al., 2012; Abbasova, 2015; Bodera et al., 2015). An interesting fact is that non - thermal effects of microwave radiation on pro- and antioxi-

doi.org/10.29228/jlsb.4

dant parameters of various tissues have been found. In particular, we have shown that exposure of the rats to microwave irradiation (from a mobile phone) up to 4 weeks for 20 min a day causes shifts in the intensity of tissue respiration of the brain structures, correlating with changes in the rate of formation of reactive oxygen species in mitochondria and lipid peroxidation intensity (Gadzhiev et al., 2016). In our other experiments, we observed the intensity-dependent pro- or antioxidant effects of EMR at 460 MHz in the lens of the eyes in the rats (Musaev et al., 2009). This effect was observed in the range of non-thermal or low intensity.

As for the effect of EMR in microwave range on the antioxidant agents of the cell, Yurekli et al. (Yurekli et al., 2006) showed widespread biological effects at a power density of 3.67 W/m^2 with specific absorption rate of 1-3mW/kg in reduced glutathione concentration in the rats. Other studies have indicated that irradiation induces reactive oxygen species (ROS), which play an important role in radiation damage of the cells (Cemek et al, 2006). The same study showed reduced glutathione (GSH) level as having antiperoxidative effect on different tissues and a scavenger effect on ROS.

The most adequate object for the study of the effects of EMR on the living organism is the blood, which, on one hand, circulates between many organs and tissues, exchanges the products of metabolism between them, including those products that arise due to the interaction of cellular structures with EMR (oxygen radicals, products of lipid peroxidation, carbonyl derivatives of proteins etc.). On the other hand, due to the high water content, the blood absorbs a significant part of electromagnetic radiation energy and transmits it to the membrane structures of blood cells and proteins of plasma. In this context, blood can serve as a source for determining the markers of EMR action on the body at the level of the oxidant-antioxidant relationship system. It is of great interest to identify qualitative and quantitative differences in this system subjected to the action of microwave EMR of intensity in the non-thermal range. This paper aims to study the concentration of lipid peroxidation product malondialdehyde and total antioxidant activity of plasma and erythrocytes in the rats subjected to whole body exposure by 460 MHz EMR at a relatively high power density. Reduced glutathione content was also determined in order to evaluate antioxidant status in the blood samples of exposed rats.

MATERIALS AND METHODS

The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation (28.11.2012, protocol No.18).

Experiments were conducted on the male Wistar albino rats weighing 250−300 g, kept in normal vivarium conditions. The animals were divided into 2 groups of 10 rats in each. One group was a control group (Con), the other - for that of (relatively) high-intensity (HIE). The apparatus "Volna-2" (manufactured in Russia) was used for the whole body exposure of animals. This device is commonly used for physiotherapy in clinics, and presents a tube generator of EMR, designed for therapeutic purposes to carry out a dosed effect on the patient by an electromagnetic field with the frequency of 460 MHz (±1%) in the decimeter wave range (wavelength 65 cm). The experiments were carried out at a relatively high intensity with power density of 30 $\mu W/cm^2$ (the output power of apparatus - 60 W). Averaged over the whole body values of SAR (Special Absorption Rate) for this intensity of irradiation were estimated by temperature change calculation and obtained, respectively, 15 mW/kg. The control group of animals was exposed to sham exposure under the same conditions with the radiation source turned off. Intact animals were not involved in the study due to the lack of significant differences in measured blood parameters between them and sham group of animals in preliminary experiments. Expositions for both experimental groups were 20 min a day, 6 days a week. The experiments were set separately for 1, 2, 3, and 4-week exposure of the animals.

Lipid peroxidation was estimated by measuring the concentration of the colored complex formed by malondialdehyde with externally-added thiobarbituric acid (TBA), according to Andreyeva et al. (1988) in plasma and Suplotov et al. (1986) in erythrocytes.

The level of reduced glutathione in hemolysed blood was assessed by Ellman method (Ellman, 1999). The main principle of the method is based on the formation of a color combination as a result of interaction of the Ellman reactive (5,5 ditiobisnitrobenzoic acid) with SH groups.

The total antioxidant activity in plasma and erythrocytes was determined by the method of Goryachkovsky (1996) in which the activity was estimated by the degree of inhibition of twin-80 oxidation to malondialdehyde by ascorbic acid-ferrous system.

SPSS for Windows version 22.0 package program was used for statistical analyses of the data. Shapiro-Wilk test was used to check whether the variables for studied groups fit normal distribution. Differences between control and experimental measurements were tested using paired samples Student's criterion. Mean ± standard error values were given as the descriptive statistics and $p<0.05$ was accepted as the statistically significant value.

RESULTS AND DISCUSSION

The concentration of plasma MDA increases dramatically after a week of exposure to relatively high intensity of irradiation (Table). The excess in concentration relative to the control animals is 96.5% at $p<0.001$ by the end of first week. The MDA level gradually decreases following the increase in duration of exposure to irradiation, and after 4-week exposure reaches a level exceeding the control level by 20% ($p<0.05$).

As for the red blood cells, yet after 2 weeks of exposure the MDA level in them becomes quite high, but not stable. To the ends of the 2nd and the 4th weeks, the increases in MDA levels reached 37.8% and 32.9%, correspondingly. At 3-week exposure of irradiation, a small excess of MDA level relative to the control group has no statistical reliability.

The level of reduced glutathione (GSH) in hemolysed blood in the rats, exposed to EMR, varies depending on the duration of radiation exposure. After the first week of exposure, the level of reduced glutathione decreases compared to the control group. Despite the absence of significant changes in the level of glutathione for 2nd week of exposure, after 3 and 4 weeks of exposure, the level of glutathione is significantly lower than in the control animals. At the end of 4 weeks of exposure, the reduction in reduced glutathione levels is approximately 50% of the control value (1.03 μmol/l vs. 2.0 μmol/l in the control). Thus, with an increase in the duration of exposure to radiation, the level of the constituent element of the blood antioxidant system, glutathione, decreases, and this indicates a weakening of the organism's antioxidant defense system.

Exposure of the rats to EMR at a relatively high intensity does not lead to significant changes in the level of TAA in plasma and red blood cells. Thus, the level of plasma TAA showed a slight decrease (12%) without 95% confidence of probability for only 2 week duration of exposure from those of all studied time segments. TAA of erythrocytes have no obvious signs of dependence on the duration of irradiation.

The decrease in the concentration of GSH in the blood, when the organism is exposed to microwave radiation, seems to be associated with a decrease in its synthesis in the liver, as well as its increased expenditure during the intensification of free-radical processes throughout the organism (Tolpygina, 2012). GSH can interact directly with free radicals, neutralizing them, but its main antioxidant and other functions are realized through enzyme systems. Damage to enzymes such as glutathione reductase, glutathione peroxidase, glutathione transferase, SOD, catalase, and some others, in pathologies or under the influence of external factors, complicates or paralyzes the enzymatic pathway of removing toxic compounds-oxidants of different nature from cells and tissues. In this aspect, a decrease in GSH activity in the blood under the influence of microwave irradiation, which has an oxidative character, on the background of minor changes in total antioxidant activity of plasma and red blood cells, may occur.

There is a number of works, pointing out to the increase in the level of free radical production in organs and tissues under the effect of microwave EMR of low intensity, taking place, for example, when using mobile phones (Challis, 2005). EMRs, no matter where they occur in the frequency spectrum, are reported to cause a rise in the levels of oxygen free radicals in experimental environments in the plants (Ursache et al., 2009) and humans (Georgiou, 2010).

Table. MDA and reduced glutathione concentrations in the blood of rats exposed to EMR 460 MHz at relatively high intensity (power density of irradiation - 30 μW/cm^2, averaged over the whole body value of SAR – 15 mW/kg)

Parameters	Control	Exposed			
		1 week	2 weeks	3 weeks	4 weeks
Plasma MDA, nmol/l	7.89±0.76	15.50±0.98**	10.10±1.10*	9.63±0.98*	9.47±0.80*
Erythrocyte MDA, nmol/l	11.50±1.50	10,60±0.82	15.80±1.34*	11.95±0.30	15.32±0.83*
Reduced glutathione, μmol/l	2.00±0.50	1.60±0.40*	2.10±0.50	1.70±0.30	1.03±0.10**

* - $p<0.05$ and ** - $p<0.01$- compared to the control group

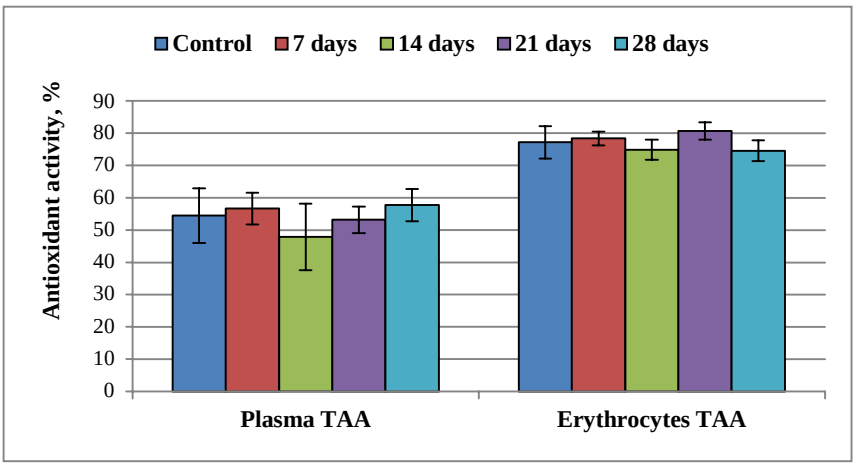

Fig. TAA levels in plasma and erythrosytes in rats exposed to EMR 460 MHz at relatively high intensity.

In the work of Elhag et al. (Elhag et al., 2007), it is indicated that EMR of cellular phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation and by changing the antioxidant activities of human blood that is leading to oxidative stress. Using EMR of 900 MHz (GSM standard), they observed an increase in the MDA concentration in the plasma of the exposed rats. At the same time, there was a decrease in the concentrations of low molecular weight antioxidants, ascorbic acid, vitamin E, reduced glutathione, as well as the activity of antioxidant enzymes catalase and superoxide dismutase. In another work (Moussa, 2009), microwave irradiation (3.5 GHz) applied for exposure of male rats also caused a significant increase in the plasma lipid peroxidation marker (MDA) while a significant decrease in glutathione concentration was observed. Results consequently suggest that the redox potential of glutathione (GSH) and nicotinamide adenine dinucleotide (NADH/NAD) were disturbed as a result of the irradiation exposure.

Bodera and others in their studies on microwave range EMR effects (5 days, 15 min/day, 1800 MHz) also showed that the levels of lipid peroxidation increase in the blood, kidneys and brain, and the total antioxidant activity of the blood at the same time becomes lower than that in the control animals (Bodera et al., 2015).

The results obtained by Moustafa et al. (2001) showed that the plasma level of lipid peroxidation significantly increased after acute exposure (up to 4 h) to the mobile phone EMR. At the same time, the activities of superoxide dismutase and glutathione peroxidase in erythrocytes showed significant reduction while the activity of catalase did not.

As shown in the work of Kumar et al. (Kumar et al., 2010), microwave radiation of extremely high frequency (10 and 50 GHz) induces significant increase in ROS production in the chronically exposed rats. The activities of serum superoxide dismutase and glutathione peroxidase decreased, and thus, the factor reduces total antioxidant capacity in the blood.

The Fenton reaction is a catalytic process that converts hydrogen peroxide, a product of mitochondrial oxidative respiration, into a highly toxic hydroxyl free radical. Some studies have supposed that EMR has another mechanism through the Fenton reaction, suggesting that it promotes free radical activity in the cells (Lai et al., 2004; Aydin et al., 2011).

There are some studies on the effect of microwave EMR on the parameters of iron in blood serum, which may indicate indirectly to the participation of Fenton's reaction in the mechanisms of free radical realization of microwave radiation in the living systems. In the work of Chetkin et al. (2017), it was shown that although chronic exposure of the rats to mobile phone irradiation does not lead to a change in the level of serum iron and ferritin, but it negatively affects both unsaturated and total iron capacities of the serum. In our previous study (unpublished result), where we used the same irradiation (460 MHz) for exposure of the rats, as in this study, we also found changes in the values of serum iron, in both unsaturated and total iron binding capacities of serum.

The biological effect of exposure to microwave EMR is a subject of particular research interest. The results of many studies not only clearly demonstrate that EMR exposure triggers oxidative stress in various tissues, but also that it causes significant changes in the levels of blood oxidant and antioxidant markers. Apparently, the risk of oxidative stress in various organs, including blood, due to electromagnetic radiation should be taken into account (Kivrak et al., 2017; Dasdag et al., 2016). However, the realization of these risks is likely to be determined by the duration of exposure to EMR and power flux density of irradiation. The risks of various disorders in the body, associated with the influence of EMR, probably depend also on the functional state of organism and additional environmental factors (Bodera et al., 2015; Yakymenko et. al., 2016; Abdolmaleki et al., 2012).

Thus, the results of our research and the data of other authors, which we have cited, allow us to expand the understanding of changes in lipid peroxidation processes and activity of antioxidant system to the effort of identification of interaction between peroxidation processes and the activation mexanism of these processes by EMR.

It seems that the generalization of the obtained experimental results can serve as a promotion for the development of methodological recommendations for primary aids in occurrence of emergency situations, associated with electromagnetic exposure.

REFERENCES

Abbasova M.T. (2015) Determination of anti-oxidant activity of blood serum in chronic body irradiation of electromagnetic radiation of 460 MHs. *Journal of Med. and Biol. Sciences (USA),* **1:** 54-56; http://bioscience.scientific-journal.com

Abdolmaleki A., Sanginabadi F., Rajabi A., Saberi R. (2012) The effect of electromagnetic waves exposure on blood parameters. *International Journal of Hematology, Oncology and Stem Cells Research*, **6(2):** 13-16.

Alghamdi M., El-Ghazaly N. (2012) Effects of exposure to electromagnetic field on some hematological parameters in mice. *Open Journal of Medicinal Chemistry*, **2:** 30-42; DOI: 10.4236/ojmc.2012.22005

Andreyeva L., Kozhemyakin L., Kishkun A. (1988) A modified thiobarbituric acid test for measuring lipid peroxidation products. *Laboratornoe delo*, **11**: 41-44 (in Russian).

Aydin B., Akar A. (2011) Effects of a 900-MHz electromagnetic field on oxidative stress parameters in rat lymphoid organs, polymerphonuclear leukocytes and plasma. *Arch. Med. Res.*, **42:** 261–267; DOI:10.1016/j.arcmed.2011.06.001.

Bodera P., Stankiewicz W., Antkowiak B. et al. (2015) Influence of electromagnetic field (1800 MHz) on lipid peroxidation in brain, blood, liver and kidney in rats. *International Journal of Occupational Medicine and Environmental Health*, **28(4):** 751-759; DOI: 10.13075/ijomeh.-1896.00255.

Cemeek M., Enginar H., Karaca T., Unak P. (2006) *In vivo* radio protective effects of Nigella sativa L oil and reduced glutathione against irradiation induced oxidative injury and number of peripheral blood lymphocytes in rats. *Photochemistry and Photobiology*, **82(6)**:1691-1696; doi.org/10.1111/j.1751-1097.2006.tb09832.x

Challis L. (2005) Mechanisms for interaction between RF fields and biological tissue. *Bioelectro-*

magnetics, **7:** 98–106; DOI: 10.1002/ bem.20119

Chetkin M., Demirel C., Kızılkan N. et al. (2017) Evaluation of the mobile phone electromagnetic radiation on serum iron parameters in rats, *African Health Sciences*, **17(1):** 186-190; DOI: 10.4314/ahs. v17i1.23.

Dasdag S., Akdag M. (2016) The link between radiofrequencies emitted from wireless technologies and oxidative stress. *J Chem Neuroanat.*, **75:** 85–93; DOI: 10.1016/j.jchemneu. 2015.09.001.

Elhag M., Nabil G., Attia A. (2007) Effects of electromagnetic fields produced by mobile phones on the oxidant and antioxidant status of rats. *Pakistan Journal of Biological Sciences*, **10(23):** 4271-4274; DOI: 10.3923/pjbs.2007. 4271.4274

Ellman A. (1999) Tissue sulfhydryl groups. *Arch. Biochem. Biophys*., **82(1):** 48-51.

Gadzhiev A., Yusifov E., Ibragimova J., Bagirova N. (2016) Study of oxygen absorption and lipid peroxidation in subcellular fractions of brain structures in rats exposed chronically to electromagnetic radiation of mobile phone. *Problems of physiology and biochemistry*, **34:** 134-142 (in Russian).

Georgiou C. (2010) Oxidative stress induced biological damage by low level EMFs: mechanism of free radical pair electron spin polarization and biochemical amplification. *Eur. J. Oncol.*, **5**: 66-113.

Goryachkovsky A. (1996) Clinical Chemistry Odessa: Astroprint, 286 p. (in Russian).

Kivrak E., Yurt K., Kaplan A. et al. (2017) Effects of electromagnetic fields exposure on the antioxidant defense system. *Journal of Microscopy and Ultrastructure*, **5**: 167-176; DOI: 10.1016/j.jmau.2017.07.003

Kumar S., Kesari K., Behari J. (2010) Evaluation of genotoxic effects in male Wistar rats following microwave exposure. *Indian J. Exp. Biol.*, **48(6):** 586-592.

Lai H., Singh N. (2004) Magnetic-field-induced DNA strand breaks in brain cells of the rat. *Environ Health Prospect.*, **112:** 687-694; DOI: 10.1289/ehp.6355

Megha K., Deshmukh P., Banerjee B. et al. (2012) Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. *Indian J. Exp. Biol*., **50**: 889–896.

Moussa S. (2009) Oxidative stress in rats exposed to microwave radiation. *Romanian J. Biophys.*, **19(2)**: 149-158.

Moustafa Y., Moustafa R., Belacy A. et al. (2001) Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. *J. Pharm. Biomed. Anal*., **26(4):** 605–608

Musaev A., Ibrahimova Zh., Gadzhiev A. (2009) Modification of experimental oxidative stress in lens tissues under effect of 460 MHz electromagnetic radiation. *Russian Journal of Physiotherapy, Balneology and Rehabilitation*, **2:** 10-13

Oktem F., Ozguner F., Mollaoglu H. et al. (2005) Oxidative damage in the kidney induced by 900-MHz-emitted mobile phone: protection by melatonin. *Arch. Med. Res*., **36:** 350-355; DOI: 10.1016/j.arcmed.2005.03.021

Suplotov N., Barkova E. (1986) Daily and seasonal rhythms of lipid peroxides and superoxide dismutase activity in erythrocytes from the inhabitants of the middle latitudes of the Far North. *Laboratornoe delo*, **8:** 459-463 (in Russian).

Tolpygina O.A. (2012) Role of glutathione in the antioxidant defense system (review). *Acta Biomedica Scientifica*, **2(2):** 178-180 (in Russian)..

Ursache M., Mindru G., Creangă D. et al. (2009) The effects of high frequency electromagnetic waves on the vegetal organisms. *Rom. Journ. Phys*., **54(1-2):** 133-145.

Yakymenko I., Tsybulin O., Sidorik E. et al. (2016) Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. *Electromagnetic Biology and Medicine*, **35(2):** 186-202; DOI: 10.3109/15368378.2015.1043557.

Yurekli A., Ozkan M., Kalkan T., Saybasili H. (2006). GSM base station electromagnetic radiation and oxidative stress in rats. *Electromagnetic Biol. and Med.*, **25:** 177-188.

Elektromaqnit şüalanmasının siçovulların qanında bioeffektləri

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Müxtəlif elektrik avadanlıqlarının və cihazların (radarlar, mobil telefonlar, tibbi avadanlıqlar) yaratdığı elektromaqnit şüalanmaları (EMŞ) insanların və heyvanların qanında biokimyəvi dəyişikliklərə səbəb olur. EMŞ-nın müxtəlif orqan və toxumalara tezlik və amplitud (intensivlik) diapazonundan asılı olaraq göstərdiyi təsirin öyrənilməsi elektromaqnit biologiyasının mühüm tədqiqat məsələlərindəndir və bununla əlaqədar olaraq insanların həyatına daha dərindən nüfuz etmiş desimetr diapazonlu EMŞ-nın bioeffektinin üzə çıxarılması məqsədi qarşıya qoyulmuşdur. Model eksperimentdə bu diapazona daxil olan 460 MHz EMŞ-nın siçovulların qanında lipid peroksidləşməsinin, ümumi antioksidant aktivliyinin (ÜAA) və reduksiya olunmuş qlütationun səviyyələrinə təsiri öyrənilmişdir. Çəkisi 250−300q olan siçovullardan 4 qrup (hər qrupda 10 baş) uyğun olaraq 1, 2, 3 və 4 həftə müddətində, həftədə 6 gün, gündə 20 dəqiqə 460 MHz EMŞ ilə xroniki şüalandırılmışlar. Şüalanma kamerasında enerji selinin sıxlığı 30 $\mu W/sm^2$ olmuşdur və şüalanmanın bu intensivliyi üçün siçovulların bütün bədəninə ortalanaraq hesablanmış xüsusi udulma gücü (SAR – specific absorbtion rate) 15 mVt/kq civarında qiymətləndirilmişdir. 4 nəzarət qrupu (hərəsində 10 baş) eyni prosedurları 1, 2, 3 və 4 həftə müddətində EMŞ mənbəyinin söndürülmüş vəziyyətində keçmişlər. 1 həftə şüalanan heyvanların qan plazmasında lipid peroksidləşməsinin məhsulu malondialdehidinin (MDA) qatılığı nəzarət qrupu ilə müqayisədə kəskin artıma ($p<0,01$) məruz qalır. Şüalanmanın sonrakı həftələrində plazmada MDA-nin qatılığının tədricən kontroldan bir qədər yüksək səviyyəyə ($p<0,05$) düşməsi müşahidə olunur. 2 və 4 həftə şüalanmış heyvanlarda eritrositlərdə MDA-nin qatılığı nəzarət heyvanlarına nisbətən daha yüksək səviyyə ($p<0,05$) göstərmişdir. Plazma və eritrositlərdə ÜAA-nin dəyişmələri kiçikdir və statistik etibarlı deyil. Reduksiya olunmuş qlütationun qatılığının şüalanmanın 4-cü həftəsinin sonuna əhəmiyyətli dərəcədə azalması aşkar edilmişdir ($p<0,01$). Nəticələr göstərir ki, hüceyrələrdə sərbəst radikal peroksidləşmə prosesləri və antioksidant müdafiə elementləri mikrodalğa tezlik diapazonunda elektromaqnit şüalanmasının bioeffeklərinin mexanizmində iştirak edir.

***Açar sözlər*:** *Elektromaqnit şüalanması, plazma, eritrositlər, lipid peroksidləşməsi, antioksidant fəallığı, reduksiya olunmuş qlütation*

Биоэффекты электромагнитного излучения в крови у крыс

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Электромагнитное излучение (ЭМИ), генерируемое различным электрооборудованием и приборами (радарами, сотовыми телефонами, медицинским оборудованием), вызывает биохимические изменения в крови людей и животных. Изучение влияния ЭМИ на органы и ткани, в зависимости от диапазона частот и амплитуд, является одним из принципиальных вопросов электромагнитной биологии, в связи с чем, выявление биоэффектов ЭМИ дециметрового диапазона, проникающего наиболее глубоко в жизнедеятельность современного человека, встает как первостепенная задача для исследования. В модельном эксперименте представленной работы изучалось влияние ЭМИ, равного 460 МГц и входящего в данный диапазон частот, на уровни перекисного окисления липидов, общей антиоксидантной активности (ОАА) и восстановленного глутатиона в крови у крыс. Из крыс, весом 250-300 г, были созданы 4 группы (по 10 голов в каждой группе), которые

подвергались хроническому облучению в течение 1-й, 2-х, 3-х и 4-х недель (6 дней в неделю по 20 минут в день) соответственно при частоте 460 МГц. В камере для облучения плотность потока мощности (интенсивность облучения) составляла 30 мкВт/см^2, удельная скорость поглощения (SAR - specific absorbtion rate), усредненная по всему телу животного, при данной интенсивности облучения была оценена на уровне 15 мВт/кг. 4 контрольные группы (по 10 голов в каждой) проходили те же процедуры в течение 1-й, 2-х, 3-х и 4-х недель при выключенном аппарате источника ЭМИ. Концентрация продукта перекисного окисления липидов малонового диальдегида (МДА) в плазме крови животных, облученных в течение 1 недели, была значительно повышена ($p<0,01$) по сравнению с контрольной группой. В последующие недели облучения концентрация МДА в плазме постепенно снижалась до уровня, несколько превышающего уровень контроля ($p<0,05$). Концентрация МДА в эритроцитах у крыс, облученных в течение 2-х и 4-х недель, оказалась достоверно выше, чем у контрольных животных ($p<0,05$). Изменения ОАА в плазме и эритроцитах были небольшими и статистически недостоверными. Обнаружено значительное уменьшение содержания восстановленного глутатиона в плазме к концу 4-й недели облучения ($p<0,01$). Результаты показывают, что процессы свободнорадикального перекисного окисления и элементы антиоксидантной защиты в клетках участвуют в механизме биоэффектов электромагнитного излучения в диапазоне микроволновых частот.

Ключевые слова**:** *Электромагнитное излучение, плазма, эритроциты, перекисное окисление липидов, антиоксидантная активность, восстановленный глутатион*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 97-103 (2020)

Morphology of lymph nodes and lymphocytes of peripheral blood during acute hypobaric hypoxia

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Accepted for publication: 15 May 2020

The article provides information about the research work carried out to study the characteristic features of morphofunctional changes occurring in the mesenteric lymph nodes and lymphocytes of peripheral blood during acute barocamera hypoxia. The study was conducted on adult male white rats with a mass of 180-200 grams. Healthy animals included in control group were not intervened, respectively acute hypoxia model was established on the II group of animals. 2 and 5 days after the experiment, the mesenteric lymph nodes were taken from the peritoneal cavity, blood from the tail vein of the animals. Mesenteric lymph nodes and peripheral blood indicators were studied using histological and morphometric methods of examination. Conducted studies have shown that in acute hypoxia, sufficient structural-functional changes of systemic character occur in lymphoid organs. At an early stage of the experiment, these changes are of a nonspecific «stress» nature, transient neutrophil leukocytosis, eosinopenia and the formation of temporary lymphopenia lead to the development of involutive-cell dystrophy, thereby initiating the first phase – mobilization phase, characterized by a decrease in adaptation intensity.

Keywords: *Acute hypobaric hypoxia, mesenterica lymph node, lymphocyte, follicle, structure*

INTRODUCTION

Hypoxia is one of the main irritant factors affecting the human body, causing the development of various pathologies, especially a number of changes in the immune system (Берова, 2007; Nikolsky, 2012). At the same time, the development of hypoxia plays a role in the formation of compensatory-adaptive reactions aimed at restoring the normal supply of tissues with oxygen. At this time, a number of biochemical reactions aimed at weakening the oxygen starvation of cells are active, and in the formation of these reactions, along with the organs of the cardiovascular and respiratory system, blood and lymphatic systems also have great importance (Alieva, 2011; Antipov, 2012; Volkov, 2015).

Regulating the interaction of organs and systems, blood is the main indicator of the internal environment of the organism, reacts immediately to oxygen deficiency. According to the authors, due to the high content of catecholamines, thyroid and corticosteroid hormones in the blood, erythrocytes from the bone marrow and blood vessels pass into the general blood circulation, resulting in the development of polycythemia (erythrocytosis), which increases the oxygen capacity of the blood. This leads to the development of long-term compensatory-adaptive reactions in the body during hypoxia of various origins (acute hypoxia, and repeated moderate-intensity hypoxia) (Khaibullina et al., 2012; Ivanov, 2014)

Leukocytes (white blood cells), especially lymphocytes, are more sensitive to the hypoxia (Kiseleva et al., 2012). Lymphocytes are not only involved in inflammation or immune response, but they also form an important system, providing homeostasis in organs, adaptation to the pathological conditions and new environments (Ivanova et al., 2014).

Despite the fact that numerous research studies have been devoted to the study of the effect of hypoxia on the immune system (Charles et al., 2019; Willard-Mack, 2006; Helbling et al., 2017),

doi.org/10.29228/jlsb.46

it is of great interest to study the morphological state of lymphoid organs (mesenteric lymph nodes and peripheral blood lymphocytes), their role in the course and development of hypoxic pathology, as well as in the formation of pathogenetic mechanisms, taking into account the high potential capabilities of immune system organs.

The aim of the study was to study the characteristic features of morpho-functional changes occurring in mesenteric lymph nodes and lymphocytes of peripheral blood in conditions of acute hypobaric hypoxia.

MATERIALS AND METHODS

To study the effect of acute hypobaric hypoxia, the experiment was conducted on 40 white rats weighing 180-200 grams. Animals are divided into 2 groups – control and experience groups. The animals included in the control group were not intervened, and the second group of experimental animals was experi¬mented in the daytime (about 10-15). To this end, they were put into the barocamera for 2 hours and created a model of acute hypoxia, 5 times a week with a break of 1 hour, 2 times a day and 2 hours every other day. In the barocamera, the tempera¬ture was 19-20ºC, atmospheric pressure was equal to the pressure 2000-3000 m above the sea level, the particles of natron lime ($Ca(OH)_2$ 81%+NaOH 3,4%+H_2O 15,6%) were used to absorb the CO_2 generated during respiration. The animals removed from the barocamera were provided with water and food and kept under control in standard vivarium conditions. On the 2nd and 5th day of the experiment, intraperitoneal anesthesia was performed by introducing 2.0-2.5% thiopental-sodium solution (100 mg/kg) into the peritoneal cavity of animals. Preparations for histological and morphometric examination were taken from the mesenteric lymph nodes and peripheral blood of decapitated animals.

Mesenteric lymph nodes and peripheral blood parameters were studied using histological and morphometric methods.

The sections of the mesenteric lymph nodes are stained with hematoxylin-eosin and covered with encrusted glass through the Canadian balm. Microscopic examination was performed under x20 and x40 magnification. Microphotography of the structural elements of the lymph nodes was performed by a digital camera of the microscope «Olympus BX-41», and morphometric parameters were calculated by Microsoft Excel computer program.

Lymphocytes of peripheral blood were studied by a binocular microscope (XSZ-107BN). The cells stained by the Romanovsky-Gimza method (Azur eosin stain) were examined in standard swabs. The leukocytic formula of the blood was calculated, leukocytes were counted, as well as morphometric analysis of lymphocytes was performed on the stained blood. The obtained morphometric indicators are (StatSoft. Inc.) calculated by the Statistica 10 computer program; statistical processing on W – Wilcoxon test (paired samples) was carried out with the control group.

Animal research was carried out in Pharmacology and Experimental Surgery departments, and the Electron Microscopy Laboratory of the Scientific Research Center of Azerbaijan Medical University. The design of the experiment was approved by the ethical committee (Protocol No. 31 of the Ethics Rules Commission and Bioethics Committee under the Ministry of Health of the Republic of Azerbaijan on 21.04.2008.).

RESULTS AND DISCUSSION

In preparations stained with hemotoxin-eosin, each lymph node is covered with the capsules. The capsule is made by numerous collagen fibers, and the smooth muscle fibers in the hilus area are also found. The capsule sends dense connective tissue septa into the lymph node to form its stroma. Beneath the capsule, there is a small subcapsular sinus, whose walls are covered by endothelial cells and including the fine-grained network formed by reticular fibers and macrophages. The subcapsular sinus is penetrated by the numerous lymph vessels, and microscopically the parenchyma of the lymph nodes appears as a dense network of the lymphatic sinuses (Fig. 1).

The lymph node consists of the cortical and medullary substance, the paracortical zone located at the border of the cortical and medullary substance. The cortical substance is made up of follicles. Numerous lymphocytes in the center of the follicles, rich in blood vessels and forming the no-

dules, attract attention. The parenchyma of the medullary substance of the lymph node consists of clusters of lymphoid tissue and medullary trabeculae formed by reticular fibers. In the paracortical zone of the lymph node, venules covered with cuboidal endotheliocytes are visualized.

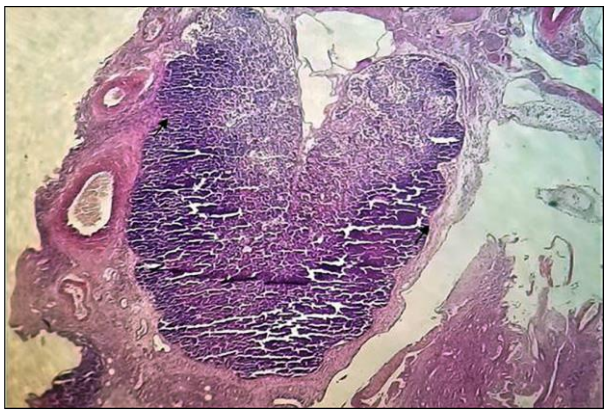

Fig. 1. Control group. Normal histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

Small lymphocytes in the cortical substance, the large lymphocytes in the medullary substance, and, medium-sized lymphocytes in the paracortical zone predominate. Histological study of lymphocytes in the peripheral blood of experimental animals included in the control group showed that the nucleus of lymphocytes repeats the shape of cells, they have a rounded shape, the size of which is within the norm (Fig. 2).

The number of lymphocytes in white blood cells of animals is 67.60±1.04%, and the number of neutrophils is 26.00±1.09% (table).

2 days after the experiment, the rats included in the II experimental group macroscopically were relatively immobile, no changes in their weight were observed. In experimental animals, weak changes in the structure of the lymph nodes are observed, morphological changes in the structure of peripheral blood lymphocytes are not detected, the nucleus of lymphocytes is round, and the size has not changed. In histological preparations, the border between the cortical and medullary substances of the mesenteric lymph nodes appears weak, the paracortical zone is not visualized, the number of lymphoid cells is reduced, in some areas lymphocytes are destructed.

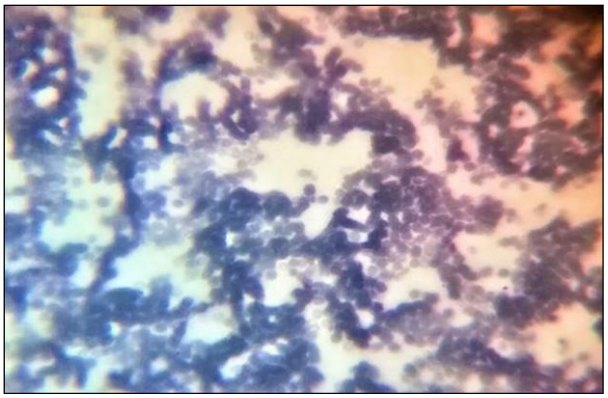

Fig. 2. Control group. Normal histological picture of peripheral blood lymphocytes. Stain: Romanovsky-Gimza x20.

In particular, the increase in the number of degranulated cells in the sinuses of the medullary substance attracts attention. Under the influence of hypoxia, there is an increase in the activity and number of macrophages in the lymph nodes of the mesenteric lymph nodes, mainly in the cavities of the sinuses. At the same time, eosinophils with a crystal structure are found in the microscope, most of which are located in the cortex. The plethoric capillaries in the microcirculatory bed are detected (Fig. 3).

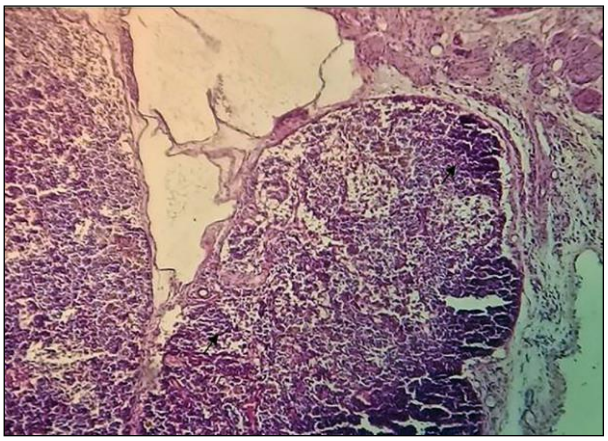

Fig. 3. 2nd day of hypoxia. Histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

Table. Parameters of leukocytic formula in peripheral b ood in norm and hypoxia *(M±m), (min-max)*

Leucocytes (L)		Control group (n)	Duration of experiment (days)	
			2nd day	5th day
Neutrophils (N)	Stab neutrophils	23.10±1.05	23.70±1.05	24.20±1.06
	Min-max	20.50-29.6	20.05-30.30	20.90-30.90
	%n	–	2.3	4.5
	%2nd day	–	–	2,1
	Segmented neutrophils	2.90±0.24	2,6±0,24	2.80±0.24
	Min-max	1.5-4.0	1.2-3.7	1.4-3.9
	%n	–	-9.6	-5.5
	%2nd day	–	–	4.6
Eosinophils		2.80±0.21	2.90±0.21	2.90±0.21
Min-max		1.9-4.4	1.99-4.49	1.93-4.43
%n		–	2.8	1.1
%2nd day		–	–	-1,7
Basophils		0.40±0.06	0.40±0.05	0.40±0.07
Min-max		0.2-0.7	0.23-0.72	0.18-0.78
%n		–	6.8	1.3
%2nd day		–	–	-5.2
Monocytes		3.20±0.33	3.20±0.33	3.20±0.33
Min-max		1.5-4.6	1.50-4.61	1.57-4.70
%n		–	0.2	2.4
%2nd day		–	–	2.3
Lymphocytes		67.60±1.04	67.20±1.03	66.6±1.01
Min-max		62.40-73.15	62.09-72.73	61.58-71.88
%n		–	-0.6	-1.5
%2nd day		–	–	-0.9
L/N		(67.60±1.04)/(26.00±1.09)	(67.20±1.03)/(26.30±1.08)	(66.60±1.01)/(26.90±1.07)

Note: n – control group; L – Lymphocytes*; N –* neutrophils*; M±m: M – average indicator of variation, m-standard error, p<0,01.*

In histological preparations, the nuclei of peripheral blood cells have a different form, the size of which has increased (Fig. 4).

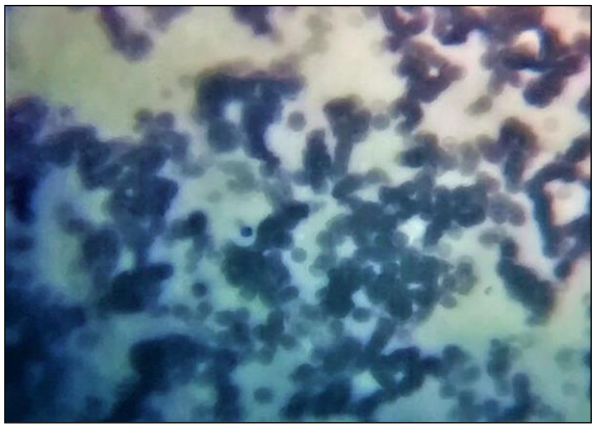

Fig. 4. 2nd day of hypoxia. Histological picture of peripheral blood lymphocytes. Stain: Romanovsky-Gimza x20.

At the same time, the number of individual populations of leukocytes in the blood, especially lymphocytes and neutrophils, was different. Thus, in the first days of the study, a decrease in the number of lymphocytes (67.20±1.03%) and a slight increase in the number of neutrophils (26.30±1.08%) was observed (tab.1). This is explained by the distribution of cell elements between lymphoid organs, circulating blood and bone marrow, as well as the transition to connective tissue.

On the 5th day of the acute hypoxia model, the examinations showed that the experimental animals were immobilized, reduced their weight, and increased their heart rate. In histological preparations, acute dystrophic and destructive changes develop in the cytoarchitectonics of morphofunctional zones of lymph nodes, as well as lymphocytes of peripheral blood. Lymph nodes grow in volume, the capsule is stretched, the parenchyma is hardened. It is impossible to distinguish the border between the cortical and medullary substances of the mesenteric lymph nodes, as well as the paracortical zone. The number and size of lymphoid nodules in the cortex of lymph nodes are reduced, and lymphocytolysis is observed (Fig. 5).

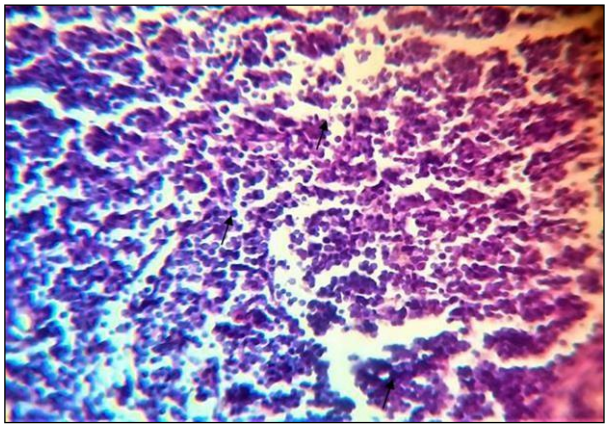

Fig. 5. 5th day of hypoxia. Histological picture of mesenteric lymph node. Stain: hematoxylin-eosin x20.

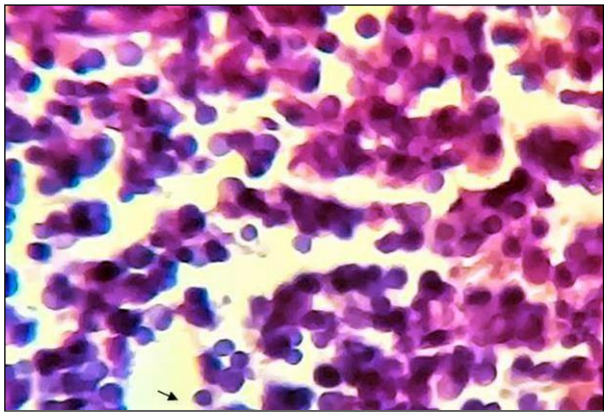

Fig. 6. 5th day of hypoxia. Histological picture of peripheral blood lymphocytes.
Stain: Romanovsky-Gimza x40.

In some areas, especially in the nuclei of lymphocytes of the medullary substance, pyknotic changes occur. Under the influence of acute hypoxia, macrophages are destroyed, destruction processes of lymphocytes are intensified, formation and increasing of the number of eosinophil granulocytes are noted. Cell changes caused by the effects of hypoxia were similar for all zones of the lymph nodes, resulting in the destruction of its reticular stroma.

At an early stage of acute hypoxia, morphological differences in the structure of lymphocytes are observed (Fig. 6). In a single volume of blood, the proliferation of lymphocytes, a decrease in large-sized lymphocytes, an increase in the number of small-sized lymphocytes and neutrophils is detected. Thus, the number of lymphocytes was 66.6±1.01%, and the number of neutrophils increased by 3.3% compared to the control group and by 2.3% compared to the second day of the experiment. This is due to an increase in the number of stab neutrophils and a decrease in the number of segmented neutrophils. In general, the number of leukocytes in the blood of animals has increased compared to the control group, which is explained by an increase in the function of neutrophils. In connection with hypoxia developed in cells, neutrophils migrate to connective tissue, regulating the activation and performance of their functions in the stromal macrophages.

The cause of changes in blood composition is the general mobilization of the body against hypoxia and stimulation of the functional activity of lymphocytes in the blood under the influence of hypoxia. On the 5th day of the experiment, the study of morphometric parameters of peripheral blood showed weak neutrophil leukocytosis and lymphocytopenia.

Thus, on the 2nd and 5th days of acute hypoxia, the quantitative indicators of lymphocytes in peripheral blood are reduced compared to the control group and are the lowest (table)

CONCLUSION

Thus, the analysis of the results of histological and morphometric studies shows that during acute hypoxia, sufficient morphofunctional changes occur in the lymphoid organs of a systemic character. These changes in the lymphoid organs are of a nonspecific "stress" nature, transient neutrophil leukocytosis, eosinopenia and the formation of transient lymphopenia lead to the development of involutive-cell dystrophy, thereby initiating the first phase – mobilization phase, characterized by a decrease in adaptation intensity. Changes in the number and morphological structure of leukocytic cells in the course of the experiment are a general mobilization of the body's protection against physical, chemical and biological factors, as well as stress factors, and can be considered an indispensable investigation to evaluate the nonspecific adaptive reaction.

REFERENCES

Alieva E.G. (2011) Morphofunctional features of the Central mesenteric lymph node of rats under antenatal antigen exposure. *Ukrainian morphological almanac*, **9(3):** 11-12 (in Russ.).

Antipov N.V. (2012) Morphological features of lymph nodes. *Ukrainian morphological almanac*, **10(2):** 3-5 (in Russ.).

Berova M.O. (2007) Immunological aspects of the body's response to hypoxia in different age periods. *Medical Sciences. Clinical medicine*, **№ 1:** 83-91 (in Russ.)).

Caldwell C.C., Kojima H., Lukashev D. et al. (2001) Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. *The Journal of Immunology*, **167(11):** p.6140-6149.

Helbling R., Conficconi E., Wyttenbach M. et al. (2017) Acute nonspecific mesenteric lymphadenitis: More than (no need for surgery). *Biomed. Research International*, **2017:** Article ID 9784565, p.1-4.

Ivanov A.B. (2014) Study of immunity indicators as a result of adaptation to hypoxia in mountain conditions and after a course of interval hypoxic training. *Izvestiya Kabardino-Balkar Scientific Center of the Russian Academy of Sciences*, **5 (61):** 250-253 (in Russ.).

Ivanova E.A., Koplik E.V. (2014) Morphological features of mesenteric lymph nodes in rats with different behavioral activity under stress. *Academic Journal of Western Siberia*, **10 (№ 1 (50)):** 58-59 (in Russ.).

Khaibullina Z.R., Vakhidova N.T. (2012) The state of peripheral blood in acute hypoxia in the experiment. *Medicine: Challenges of today: materials of the international journal*. Science Conf. Chelyabinsk: Two Komsomolets, p. 24-29 (in Russ.).

Kiseleva N.M., Kuzmenko L.G., Nkane Nkoza M.M. (2012) Stress and lymphocytes. *Pediatrics*, **91(№ 1):** 137-143 (in Russ.).

Nikolsky I.S. (2012) Reaction of blood system on hypoxic hypoxia and hematopoietic stem cell circulation. *Environment & Health*, **2:** p.79-80.

Volkov V.P. (2015) On the issue of morphometric assessment of functional immunomorphology of lymph nodes: a review of the literature and a new algorithm. *Universum: Medicine and pharmacology*, **№7-8 (19):** 15 p. (in Russ.).

Willard-Mack C.L. (2006) Normal structure, function and histology of lymph nodes. *Toxicol. Pathol.*, **34(5):** 409-424.

Kəskin hipobarik hıpoksiya zamanı müsariqə limfa düyünlərinin və periferik qanın limfositlərinin morfologiyası

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Məqalədə kəskin barokamera hipoksiyası zamanı müsariqə limfa düyünlərində və periferik qanın limfositlərində baş verən struktur-funksional dəyişikliklərin səciyyəvi xüsusiyyətlərinin öyrənilməsi məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir. Tədqiqat 2 qrup üzrə ayrılmış kütləsi 180-200 qram olan yetkin erkək ağ siçovullar üzərində aparılmışdır. I kontrol qrupuna daxil edilən sağlam təçrübə heyvanlarına müdaxilə edilməmiş, II qrup üzərində kəskin hipoksiya modeli yaradılmışdır. Eksperimentdən 2 və 5 gün sonra heyvanların periton boşluğundan müsariqə limfa düyünləri, quyruq venasından qan götürülmüşdür. Müsariqə limfa düyünləri və periferik qanın göstəriciləri histoloji və morfometrik müayinə metodlarından istifadə etməklə öyrənilmişdir. Aparılmış tədqiqatlar göstərmişdir ki, kəskin hipoksiya zamanı limfoid orqanlarda sistemli xarakter daşıyan kifayət dərəcədə struktur-funksional dəyişikliklər baş verir. Limfoid orqanlarda baş verən bu dəyişikliklər qeyri-spesifik «stress» xarakteri daşıyır, keçici neytrofil leykositoz, eozinopeniya və müvəqqəti limfopeniyanın meydana gəlməsi involyütiv-hüceyrə distrofi-

yasının inkişafına səbəb olur, bununla da uyğunlaşma intensivliyinin azalması ilə xarakterizə olunan ilk faza – səfərbərlik fazası başlanır.

Açar sözlər: *Kəskin hipobarik hipoksiya, müsariqə limfa düyünü, limfosit, follikul, struktur*

Морфология лимфоцитов брыжеечных лимфоузлов и периферической крови при острой гипобарической гипоксии

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В статье представлена информация об исследовательской работе, проведенной с целью изучения характерных особенностей структурно-функциональных изменений в брыжеечных лимфоузлах и лимфоцитах периферической крови при острой барокамерной гипоксии. Исследование проводилось у взрослых белых крыс-самцов весом 180-200 грамм, разделенных на две группы. Здоровые подопытные животные, входящие в I группу контроля, не подвергались вмешательству, у II группы подопытных животных была создана соответствующая модель острой гипоксии. Через 2-е и 5-ро суток после эксперимента у подопытных животных была взята кровь из хвостовой вены и из брыжеечных лимфатических узлов в брюшной полости. Показатели крови из брыжеечных лимфатических узлов и периферической крови изучались с использованием методов гистологического и морфометрического обследования. Проведенные исследования показали, что при острой гипоксии в лимфоидных органах в колоссальной степени происходят структурно-функциональные изменения, имеющие системный характер. На ранней стадии эксперимента эти изменения носят неспецифический «стрессовый» характер, а появление переходного нейтрофильного лейкоцитоза, эозинопении и временной лимфопении становится причиной развития инволютивно-клеточной дистрофии, что в свою очередь дает начало первой фазе – фазе мобилизации, характеризующейся снижением интенсивности адаптации.

Ключевые слова: *Гипобарическая гипоксия, брыжеечный лимфоузел, лимфоцит, фолликул, структура*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 104-109 (2020)

Peculiarities of involutory processes (aging processes) on background of light desynchronization

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Accepted for publication: 12 June 2020

To identify the effect of light desynchronization on the neuroendocrine regulation of physiological processes in the development of aging, we studied the effect of light desynchronization on the degree of involutive processes (aging processes). To objectify the results of the effect of light desynchronization on the neuroendocrine regulation of physiological processes in the development of the aging organism, the parameters were evaluated before light desynchronization, on the 1st day after the light desynchronization, on the 12th and 23rd days after the light desynchronization. Since the regulatory mechanisms of the organism have pronounced biological rhythms, one of these factors is light desynchronization. Desynchronization occurs due to light pollution of environment, as well as among the people who make transmeridional flights and work on a night shift for realization of professional duties. Light desynchronization can cause not only physiological, but also psycho-emotional disorders in healthy people and the development of premature aging of the whole organism and the early development of age-associated conditions.

Keywords: *Desynchronization, involutory processes, light desynchronization, interleukin-1, interleukin-4, Ki-67 protein*

INTRODUCTION

Still exist information deficiency about neuroimmune and endocrine changes accompanying the light desynchronization - one of the signs of the dysfunction of central nervous system (Комарова, 1989; Романов, 2000; Анисимов, 2014). To the data of experimental studies, the changes of the homeostasis of a number of signal molecules, including glucocorticoids, hormones of hypothalamic-pituitary-adrenal system, serotonin, melatonin, endogenic opioids, as well as proinflammatory cytokines (Гончарова**,** 2010). Neuroendocrine status of organism usually is defined with the activities of glucocorticoid receptors regulating the functional activity of all chains of immunity.

The biological role for glucocorticoids concludes in regulation of intracellular metabolism and functions of the genetic apparatus of the cells. These hormonal-metabolic interrelations increase the organisms' resistance to stresses that occur due to light desynchronization (Костенко, 2013; Зарипов, 2015). The above-mentioned issues make grounds for the studies of the influence of light desynchronization to neuroendocrine regulation of physiological processes at the organisms aging through evaluation of the mechanisms of the changes of glucocorticoid reception in experimental studies.

MATERIAL AND METHODS

The studies were conducted on the albino nonlinear mice with mass of 17.8±0.04-21.5±0.04 g, which were culled into two groups: young (average age 10.9±0.01 week, n=78) and old (average age 19.1±0.01week, n=82) mice. Body mass of mice was determined on the laboratory scales Sartorius ED423S-RCE (Germany).

Light day lasted 12. Food and water were available to the animals ad adlibitum.

For objectification of the results the decision of investigating the influence of light desynchronization on neuroendocrine regulation of physiological processes at the course of organisms aging was

doi.org/10.29228/jlsb.47

done. We have conducted the evaluation of parameters period to conduction of light desynchronization, at the 1st day after conduction of light desynchronization, and at the 12th and 23rd days after cessation of light desynchronization. The first half of each rhythm is favorable (positive phase), the second half is unfavorable (negative phase.

The indexes to the 23rd days the sacrificed considered more objective for evaluation of full action of light desynchronization to neurohumoral regulation of physiological processes at the development of organism aging.

After 23 days mice were sacrificed and the results obtained prior to the beginning of light synchronization were accepted as a control, because the animals were kept under natural light regime.

Five minutes before conducting the manipulations (taking the blood samples) animals were anesthetized with intramuscular injection of mixture of Telasol (Zoetis Inc, USA) ata dose of 0,1 ml/kg and Xylanit (Nita-Farm, Russia) ata dose of 0,1 ml/kg.

Light desynchronization was modeled through of the changing of light regime in the laboratory. Animals have been under gone to the influence of the combination of natural and at night hours, of artificial elucidation provided by the luminescent lamp, equivalent to filament lamp of the intensity 60 Watt. For the evaluation of immune status, we measured the levels of tumor necrosis factor alpha, interleukin-1, interleukin-4 and the ratio of CD4/CD8 expressions.

The method of evaluation the levels of interleukins was based on solid phase "sandwich"-type of the ELISA -test. The specific reagents of the set for the ELISA-test are the monoclonal antibodies to the studied interleukin, adsorbed on the surface of the wells of polystyrene plate. Protein Ki-67 is the widely recognized and used marker of proliferation, expressed in all kinds of tissues. The aging process is characterized with reaching the Hayflick limit and decrease or total termination of the cells capabilities to division.

Upregulation of this marker demonstrates development of the pathological reactions in the organism. In this relation Ki-67 protein may be an important marker for evaluation of the decreasing of cell's proliferative activity and level of involutory processes in the studied organs.

Decreasing the increased expression of Ki-67 protein, which is initiated at the background of stress stimuli of any origin, indicates to the existence of the physiological abilities of the organism to adaptation, to the normal physiological status of the antioxidant system, to the higher regenerative ability, as well as to the capabilities to decrease physiologically the level of involutory processes (aging processes) in the organism (Buondonno, 2019).

23 hours later the mice were sacrificed. As control additionally 10 young mice prior to conducting the light desynchronization were taken additionally, 10 young mice at the 1st day after conducting of light the cuts of the brain prepared. The desynchronization and 10 young mice at the 12th day after conducting the light desynchronization; 10 old mice till conducting the light desynchronization, 10 old mice at the 1st day after the light desynchronization and 10 old mice at the 12th day after the light desynchronization.

Then we have prepared cuts of the brain. Parts of the brain was placed in the 10% solution of paraformaldehyde at phosphate buffer (PBS pH=7.3) for 24 at a temperature 4ºC. We have made cuts of thickness of 20 mcm with the application of manufactured by Leica model of CM 1510S, (Germany). Then cuts were placed on the slides and were stained with hematoxylin and eosin.

We have determined the changes of the expressions of nuclear antigen Ki-67, on the brain cuts of young and old mice after the influence of light desynchronization. The antigens Ki-67, was chosen for analyses, because it is present at all stages of cell cycle, besides G0, and is a marker of the cell proliferation.

Characteristics of the used antigens Cell Marque Ki-67 (Cell Marque, США, Positive control slides: 275S):

- Clone: SP6, rabbit
- 0.1 ml Conc.: 275R-14
- 0.5 ml Conc.: 275R-15
- 1.0 ml Conc.: 275R-16
- 1.0 ml Predilute: 275R-17
- 7.0 ml Predilute: 275R-18

For our studies we have used microscope Olympus IX81. Microscope was provided with the digital camera Olympus DP72 (Japan), which is joined to personal computer. Measuring of the depth of outer nuclear layer (ONL) was conducted at microphotographs of stained cuts.

RESULTS AND DISCUSSION

The results of immune histochemical reactions were expressed as of percent ratios of the stained cells to the total amount of cells at preparation. Protein Ki-67 is a generally recognized and widely used marker of proliferation, expressed at all kinds of tissues.

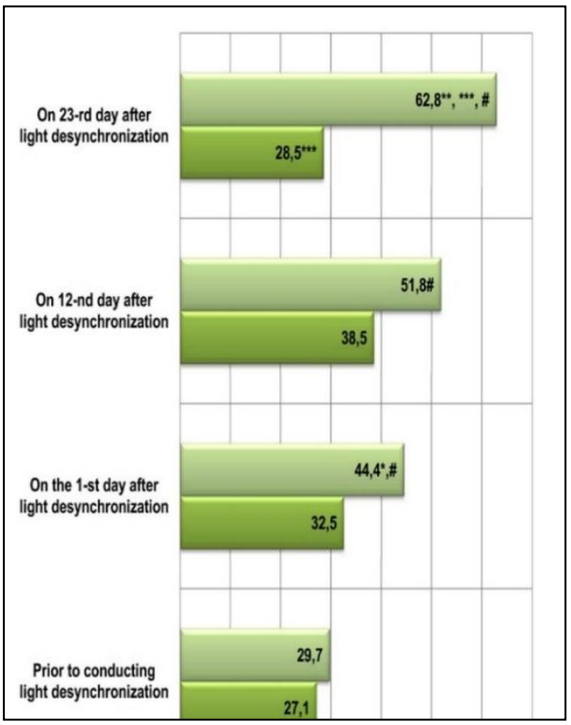

Figure 1. The dynamics of expression of Ki67.

* p<0.05 between indexes before conducting of light desynchronization and at the 1st day after light desynchronization;

** p<0.05 between indexes at the 12th day after conducting of light desynchronization and at the 23rd days after light desynchronization;

*** p<0.05 between the indexes before conducting light desynchronization and at the 23rd day after light desynchronization;

# p<0.05 between indexes of the young and old mice.

Process of aging is characterized with reaching to the Hayflick limit and decreasing or totally cessation of cell capabilities to division. Increasing of its level effects advent of the pathological reactions in the organism.

In this relation protein Ki-67 may be an important marker for evaluation of decreasing of the proliferating activity of the cells degree of involutory processes of the studied organism (picture 1, 2).

At the figure 2 the brain cuts, stained with hematoxylin and eosin at the 23rd days after conducting of light desynchronization at young and old mice are presented. Much mice predominance of the expression area of the nuclear antigen Ki-67 on the old mice than on the young ones, is observed.

We have determined the changes of the expression of the nuclear antigen Ki-67, which is present at the all stages of cell cycle, besides G0, at the brain cuts in the young and old mice after the effect of light desynchronization.

So, the expression area of the nuclear antigen Ki-67 at the young mice prior to conducting light desynchronization made of 27,1±0,3%, that is related to physiological expression of given marker. It means, that the degree of involutory processes (aging processes) was with in at the physiological limits and was accepted for the point control on the young mice.

After conducting light desynchronization the expression area of Ki-67 antigen at the young mice on the first day slightly increased to 32.5+1.1 %, p>0,05 between indexes prior to conducting light desynchronization and on the 1st day after conducting light desynchronization; but it does not reach to reliable differences, demonstrates of induction of normal physiological reactions of the organism ward adaption in response to the effect of light desynchronization and of degree of involutory processes (aging processes) with in the physiological limits in the young ages.

Furthermore at the 12th day the expression area of the nuclear antigen Ki-67 on the young mice slightly increased up to till 38.5±0.7 %, by 1.2 times compared to the index on the 1st day and by 1.4 times in comparison to the control level the, p>0,05 between indexes at the 1st day after conducting light desynchronization and at the 12 days after conducting the light desynchronization, p<0,05 between indexes till conducting light desynchronization and on the 12th day after conducting light desynchronization, reflects the induction of the normal physiological reaction of the organism to adaptation in response to the effect of light desynchronization and the degree of involutory processes (aging processes) with in the physiological limits at the young ages.

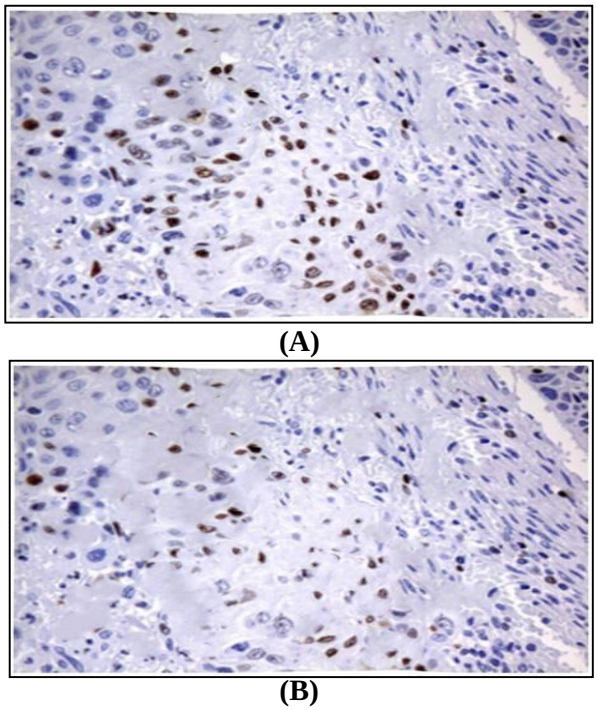

Figure 2. Expression of Ki-67 in the brain. Stained with hematoxylin and eosin X 400.
A- Young mice on the 23rd - days after light desynchronization; B- Old mice on the 23rd - days after light desynchronization

To the 23rd day the expression area of the nuclear antigen Ki-67 at the young mice was recovered to 28.5±0.3 %, p<0.05 between the indexes on the 12th day after conducting the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between indexes prior to conducting the light desynchronization and at 23rd day after conducting the light desynchronization, that proves existing of adequate physiological capabilities on the young ages to adaptation of organism on the 23rd day to stressful stimuli in the form of a light desynchronization and the degree of involutory processes (aging processes) with in the physiological limits in the young ages.

The expression area of the nuclear antigen Ki-67 on the old mice prior conducting the light desynchronization made 29.7±0.3%, that really does not differ from the expression area of the nuclear antigen Ki-67 on the young mice and refers to the physiological expression of the given marker, that means the degree of the involutory processes (aging processes) was within the physiological limits and was accepted as a control point on the old mice.

After conducting the light desynchronization the expression area of nuclear antigen Ki-67 in the old mice at the first days was significantly increased up to 44.4+1.5 %, p<0,05 between the indexes conducting of light prior to desynchronization and on the 1st days after the light desynchronization, indicating to induction of pathological insufficient physiological reaction for the organism to adaptation as response to the effects of light desynchronization and to the increased degree of involutory processes at the old ages after the effects of light desynchronization.

Furthermore on the 12th day the expression area of the nuclear antigen Ki-67 at the old mice obviously slightly up to 51.8±1.7 %, by 1.2 times compared to the index on the 1st day and by 1.7 times compared to the control point, p>0,05 between the indexes on the 1st day after light desynchronization and on the 12th day after the light desynchronization, p<0,05 between the indexes prior to conducting light desynchronization and on the 12th day after light desynchronization, p<0,05 between the indexes on the young and old mice that reflects induction of the pathological in sufficient physiological reaction for the organism toward adaptation in response to the effect of the light desynchronization and increasing the level of involutory processes (aging processes) after the effect of the light desynchronization at the old ages.

To the 23rd day the expression area of the nuclear antigen Ki-67 on the old mice increased up to 62.8±1.8 %, p<0,05 between the indexes on the 12th day after the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between the indexes prior to conducting the light desynchronization and on the 23rd day after the of light desynchronization, p<0.05 between the indexes on the young and old mice, that proves the existence of the pathological (insufficient) physiological reaction of the organism toward adaptation as an on organisms response to the effect of light desynchronization and of the increasing level of involutory processes (aging processes) at the old years after the effect influence of light desynchronization (Hoffman, 2006).

Downregulation of the increased expression of Ki-67 protein which occurs at the background of stress stimuli of any origin, indicates to the about presence of physiological potential of the organism toward adaptation, to the normal physiological status of antioxidant system, high regenerative abilities, as well to the abilities to decrease physiologically the degree of involutory processes (aging processes) in the organism.

CONCLUSION

We have determined the changes of the expression of the nuclear antigen Ki-67, which is presented at the all stages of cell cycle besides G0, at the brain cuts on the young and old mice after the effects of light desynchronization.

Analysis of the dynamics of the expression areas dynamics of nuclear antigen Ki-67 at young mice informed about normal physiological reaction of the organism to ward adaptation as response to influence of light desynchronization and the degree of the involutory processes (aging processes) with in the physiological limits on the young ages.

Analysis of the dynamics of the expression areas of the nuclear antigen Ki-67 on the old mice showed pathological (insufficient) physiological reaction of the organism forward adaptation as response to the effects of light desynchronization and up regulation of the degree of involuntary processes (aging process) in old ages after the effect of light desynchronization.

REFERENCES

Анисимов В.Н., Виноградова И.А., Букалев А.В. и др. (2014) Световой десинхроноз и риск злокачественных новообразований у лабораторных животных: состояние проблемы. Вопросы онкологии. **60(14):** 15-27.

Гончарова Н.Д. (2010) Гипоталамо-гипофизарно-адреналовая система у лабораторных животных: циркадные ритмы, стресс-реактивность, старение. *Материалы VIII Всероссийской конференции «Нейроэндокринология»*. Санк-Петербург, с. 52-54.

Заморский И.И., Пишак В.П. (2003) Функциональная организация фотопериодической системы головного мозга. *Успехи физиологических наук*, **34(4):** 37-53.

Зарипов А.А., Янович К.В., Потапов Р.В., Корнилова А.А. (2015) Современные представления о десинхронозе. *Современные проблемы науки и образования*, **№3:** 8 с.

Костенко, Е.В., Маневич Т.М., Разумов Н.А. (2013) Десинхроноз как один из важнейших факторов возникновения и развития цереброваскулярной заболеваний. *Медицина и здравоохранение*, **№2:** 104-116.

Bahadoran Z., Mirmiran P., Jeddi S. et al., (2018) The nitrate-nitrite-nitric oxide pathway: Findings from 20 years of the Tehran lipid and glucose study. *Int. J. Endocrinol. Metab*., **16 (4, Suppl):** e84775, 6 p.

Buondonno I., Sassi F., Carignano G. et al. (2019) From mitochondria to healthy aging: The role of branched-chain amino acids treatment: MATeR a randomized study. *Clin. Nutr*., **1:** 261-269.

Hoffman M.A., Swaab D.F. (2006) Living by the clock: The circadian pacemaker in older people. *Aging Res. Rev*., **5:** 33-51.

İşıq desinxronlaşması zamanı involyutiv proseslərin (qocalma proseslərinin) xüsusiyyətləri

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Orqanizmin qocalmasının inkişafında fizioloji proseslərinin neyroendokrin tənzimlənməsinə işıq desinxronlaşmasının təsiri öyrənilmişdir. Alınan nəticələrinin obyektivliyi üçün biz orqanizmin qocalmasının inkişafında fizioloji proseslərinin neyroendokrin tənziminə işıq desinxronlaşmanın təsirini öyrənməkdən ötrü nəzərə aldığımız parametrlər işıq desinxronlaşmasından qabaq , işıq desinxronozu tətbiq edildiyindən 1 həftə sonra, 12-ci və 23-cü həftələrdə təyin edilmiş və qiymətləndirilmişdir. Orqnizmin tənzimləyici mexanizmləri aydın ifadə olunan bioloji ritmlərə malikdir,ritmləri pozan faktorlardan biri də işıq desinxronlaşmasıdır. Desinxronlaşma ətraf mühitdə işıqlılıq şəraitinin dəyişilməsi, həmçinin transmeridial təyyarə uçuşlarını həyata keçirən şəxslərdə və peşə zəruriyyəti ilə əlaqədar gecə növbəsində işləyən işçilərdə baş verir. İşıq desinxronlaşması sağlam adamlarda tək emosional pozuntuları doğura bilər və bütün orqanizmin erkən qocalmasının inkişafının və yaş-assosiyalaşmış vəziyyətlərinin erkən inkişafının səbəbi ola bilər.

Açar sözlər: *Desinxronoz, involyutiv proses, işıq desinxronozu, interleykin-1, interleykin-4, Ki-67 zülalı*

Особенности инволютивных процессов (процессов старения) при световой десинхронизации

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Для выявления влияния световой десинхронизации на нейроэндокринную регуляцию физиологических процессов в развитии старения организма нами были проведены исследования, цель которых заключалась в изучении влияния световой десинхронизации на степень инволютивных процессов (процессов старения). Для объективизации результатов влияния световой десинхронизации на нейроэндокринную регуляцию физиологических процессов в развитии старения организма оценка параметров была проведена до световой десинхронизации, в 1–е сутки после проведения световой десинхронизации, на 12-е и 23-е сутки после проведения световой десинхронизации. Поскольку регуляторные механизмы организма имеют выраженные биологические ритмы, одним из таких факторов является световая десинхронизация. Десинхронизация происходит вследствие светового загрязнения окружающей среды, а также у лиц, совершающих трансмеридиональные перелеты и, в силу профессиональной необходимости, работающих в ночную смену. Световая десинхронизация может вызывать не только физиологические, но и психоэмоциональные расстройства у здоровых людей и стать причиной развития преждевременного старения всего организма и раннего развития возраст-ассоциированных состояний.

Ключевые слова: *Десинхронизация, инволютивные процессы, световая десинхронизация, интерлейкин -1, интерлейкин -4, белок Ki-67*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 110-115 (2020)

The role of neurochemical systems of the brain in the regulation of the hippocampal theta-rhythm

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Accepted for publication: 03 April 2020

In chronic experiments on the rabbits it has been shown that electric destruction of the dorsal amygdalo-fugal pathway leads to complete and persistent blockade of hippocampal theta rhythm in contrast to the ventral amygdalo-fugal pathway. In intact animals, electro- and chemostimulations of various limbic structures of the brain (amygdala, hypothalamus, reticular formation, medial septum nucleus) lead to the formation of well pronounced theta rhythm in the hippocampus, but after destruction of the dorsal amygdalo-fugal pathway no theta-rhythm in this structure was observed. Restoration of hippocampal EEG took place under the intra-hippocampal application of carbocholine and strychnine. It is proposed that one of the necessary conditions for the regulation of excitability of hippocampal neurons is the integrity of the dorsal amygdalo-fugal pathway through which the regulatory influences of the amygdala on the hypothalamic neuro-secretory cells are realized.

Keywords: *Hippocampal theta-rhythm, dorsal and ventral amygdalo-fugal pathways, electrical and chemo-stimulation, destruction*

INTRODUCTION

For many years, one of the controversial issues in the electrophysiology is the study of the hippocampal theta rhythm. The medial septum nucleus, standing at the entrance to the hippocampus, demonstrates the importance of education (Kichigina, Kutyreva 2002; Kitchigina, 2006; Kitchigina, Popova, Sinelnikova, Malkov, Astasheva, Shubina, Aliev, 2013; Mysin, Kitchigina, Kazanovich, 2015). In addition to the data, indicating to the pacemaker role of the septum, there are works showing a definite role of stem-diencephalon structures in the mechanisms of formation of hippocampal theta rhythm: a great importance is given to the reticular formation (Steriade, 1996), hypothalamus (Smythe,1991), thalamus (Smythe, 1991), locus cereleus (Berridge, Espana, 2000) and nucleus raphe (Kitchigina, 2006).

Recently it has been shown that the medial septum nucleus receives phases of the already encoded information from the uplink system, whose frequency determines frequency of the discharges of the septal hippocampal theta rhythm. There is evidence that this information comes from the supra-mammillary nucleus of the hypothalamus (Vertes, 1992).

Our earlier researches has shown that destruction of the dorsal amigdalo-fugal pathway (DAP), in contrast to destruction of the ventral amygdalo-fugal pathway (VAP), results in complete and irreversible blockade of hippocampal theta rhythm (Gasanov, Kasimov, Bagirova,1989). To clarify the reasons for the profound changes we have conducted electric and chemo-stimulation of the limbic structures of the brain (amygdala, hypothalamus, reticular formation, medial nucleus of the septum, hippocampus) before and after destruction of the DAP.

METHODS

Experiments were carried out on 16 mature rabbits having body mass 2.5-3.0 kg. Both recording the electrical activities from the hippocampus and septum and collection of the samples for morphological studies were performed 18-27 days later from such destruction. The EHipG was recorded from the dorsal hippocampus (the CA1field:

doi.org/10.29228/jlsb.48

P 3.0, L 2.0, H 18.0, and the CA3 field: P 0 2.0, L6.0, H17.0) and from the medial nucleus of the septum (A-3.0; L0.5; H10.5) on the encephalograph Medikor EEG-16E with the use of needle electrodes electrically insulated except for the tip. Spectral EHipG analysis was performed using standard electro-encephalographic approaches. Test substances were strychnine (1%), carbachol (0.5–1.5 μg), serotonin (5-50 μg), and noradrenaline (15-20 μg), applied in a volume of 5-6μL via a chemotrode, implanted into the field CA3. Electrocoagulation of the dorsal amygdalo-fugal pathway (stria terminalis) was performed using electrodes implanted in the pre-commissural area (A-1, L5, H18) with currents of up to 1.0 mA for 15–25 sec. Electrical stimulation of the extra-hippocampal structures (reticular formation: P9, L2.5, H18.2; basolateral nucleus of the amygdala: A-1, L5, H18; central nucleus of the amygdala: A-1,L5, H16; supraoptic nucleus of the hypothalamus: A-3, L2.2, H15.8; ventro-medial nucleus of the hypothalamus: AP0,L0.5, H17; medial mammillary nucleus of the hypothalamus: P2, L0.5, H18.5) and field CA3 was performed using an ÉSU-1 stimulator with square-wave impulses at frequencies of 5–100 per sec, amplitude 2–4 V, and duration 0.15 msec, for 15–30 sec; a histogram method was used for amplitude-frequency analysis of the EEG, as described Fujimori (Fujimoriet al., 1958).

RESULTS AND DISCUSSION

The results of the experiments showed that the baseline hippocampal and septal EEGs demonstrate irregular activity dominated by oscillations the range of 4–6 Hz. Comparison of the electrical activity of the hippocampus and different fragments of the conditioned reactions supports the existence of a marked correlation of the theta rhythm with such forms of behaviors as resting, voluntary locomotion, jumps and runs, and licking, being in agreement with the results obtained in our previous studies (Gasanov, Kasimov, Bagirova,1989). Application of the test substances to the dorsal hippocampus before lesioning of the stria terminalis led to ambiguous results. In particular, the effects of biogenic monoamines ultimately led to a redistribution of the peak of the EEG amplitude frequency characteristic in the hippocampus. In particular, while serotonin increased the EEG in the region 5–6 Hz, noradrenaline displaced the peak of the frequency characteristic to the region 4–5 Hz. The effects of strychnine and carbachol were significantly different. In this experimental situation, the application of carbachol (like strychnine) resulted in a generation of high-amplitude, regular theta waves of frequency 6–7.5 Hz at different time points, which with time course could transform into epi-discharges (Fig. 1, III, IV and V). The EEG changes seen after the applications of carbachol and strychnine started in all outputs simultaneously and were seen for prolonged periods of time (the maximum observation period was 3 h).

The destruction of the dorsal amygdalo-fugal way unlike to the destruction of ventral amygdalo-fugal way leads to a complete and irreversible blockade of hippocampal theta rhythm (Fig. 2, II and III). Dynamic observations of the EEG after unilateral lesioning of the stria terminalis showed that the onset of EEG depression often started before the transition period, which showed transient epileptiform activity which subsequently disappeared, leaving only super slow oscillations. On this background we were unable to record neuron spike activity from the field CA3 of the hippocampus, though continuing recording resulted in the appearance of occasional neuron action potentials in the cerebral cortex. Administration of biogenic monoamines into the hippocampus after lesioning of the stria terminalis did not induce any changes at all. The electrical stimulations of the various extra-hippocampal structures (mRF, hypothalamus, amygdala) did not bring to recovery of the electrical activity of the hippocampus, while stimulation of the hippocampus itself produced only epi-discharges, when the maximal stimulating electric current was used. The effects of the applications of carbachol and strychnine were rather different. In this situation, there was a tendency to recovery of the overall activities of the hippocampus and septum, with some features consisting of short-lived (20–30 sec) periodically repeated generation of regular rhythmic activities in the range 0.6-7.5 Hz. Attention is drawn to the fact that, on one hand, recovery of the electrical activities in the hippocampus and septum occurred spontaneously in all outputs, while, on the other hand, there was a marked synchronicity in the generation of the electrical activities.

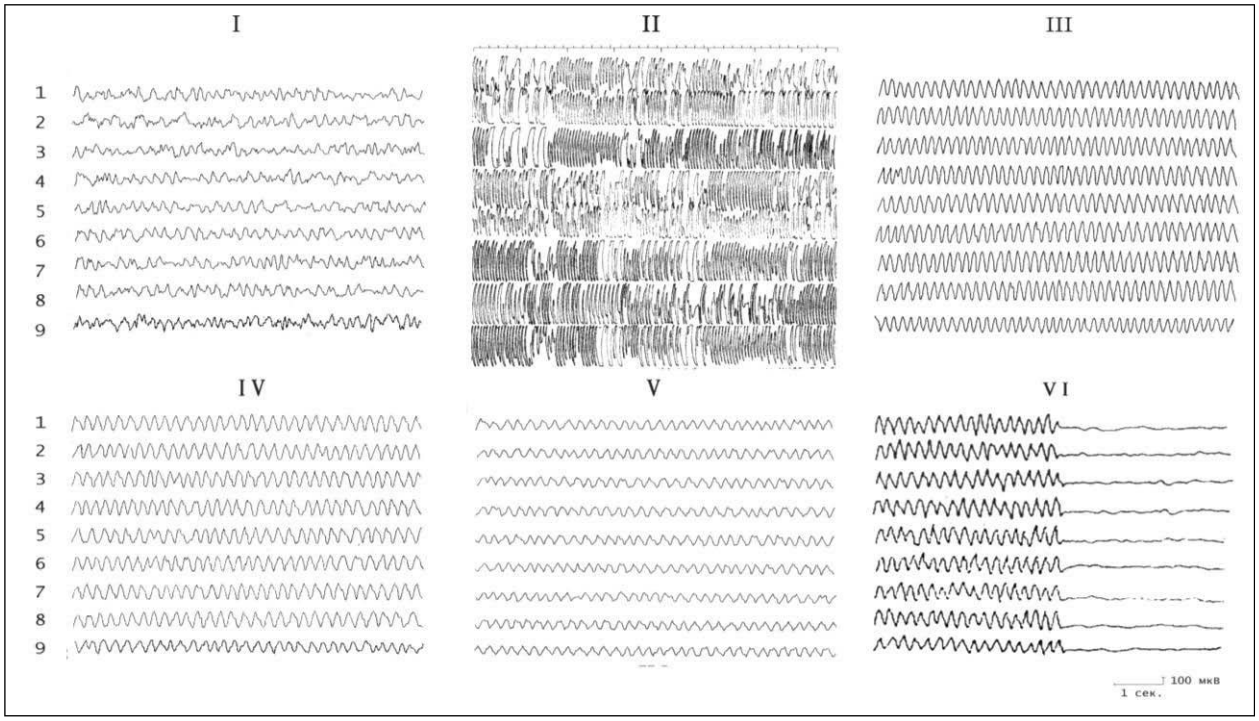

Figure 1. The influences of electric and chemical stimulations of the field CA3 of the dorsal hippocampus on the electrical activity of the hippocampus. I - baseline; II - instant electrical stimulation; III-after application of carbachol; IV-after application of serotonin; V - after application of noradrenaline; VI - application of carbachol on the background of the destruction of the dorsal amigdalo-fugal way.1,2-field CA1; 3,4-field CA3 of the ipsi- and contralateral hemispheres; 5,6 - ventral hippocampus of the ipsi- and contralateral hemispheres; 7,8 - dentate gyrus of the ipsi- and contralateral hemispheres; 9 - medial nucleus of the septum.

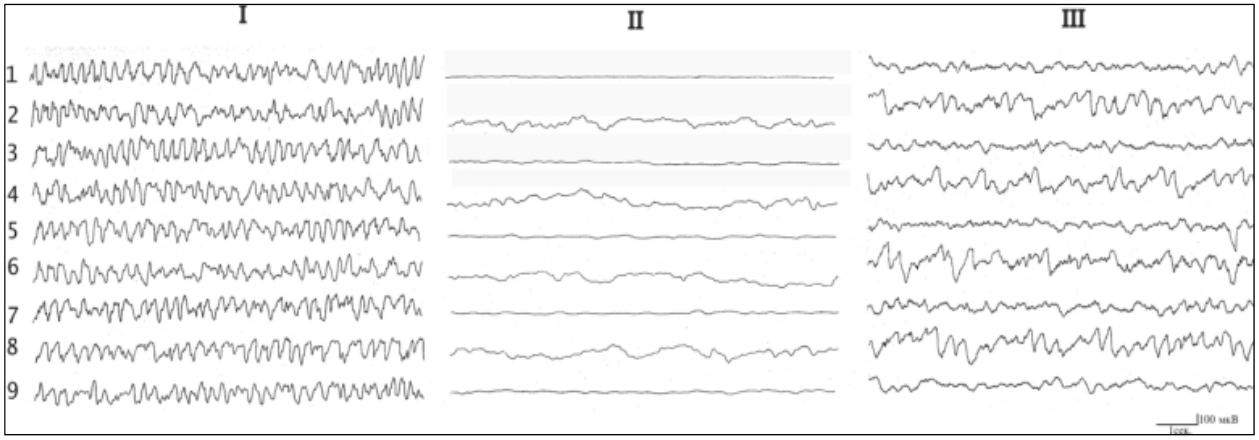

Figure 2. Changes in hippocampal electrical activity on the rabbits under destruction of the dorsal and ventral amigdalo-fugal ways. I-baseline; II-after destruction of the dorsal amigdalo-fugal way; III-after destruction of the ventral amigdalo-fugal way. The rest designations are the same as on Figure 1.

The effects of carbachol and strychnine were mostly similar and were long-lasting. In addition, analysis of the behavioral reactions provided evidence that conditioned responses persisted under the lesioning of the stria terminalis, with only one element to increase - is the latency of the response to the conditioned signal (2.0–2.5 sec as compared to 1.0–1.5 sec before lesioning). Hence, the analysis of our data allows to make a conclusion that the actions of various neurochemicals in the EEG activities of the hippocampus have a number of common and distinct properties. The former includes: 1) the occurrence of synchronized theta wave activity; 2) the absence in the different areas of the hippocampus of differentiation of the bioelectric reactions; 3) disturbances of the regularity of the theta-wave activity and the emergence of epi-discharges by increasing the doses of the studies monoamines injected into the brain structures. As for the properties that distinguished the actions of the applied neurochemical agents, they include: 1) the emergence of dominant frequency of 6-7.5 numbers/sec for the cholinergic, 5-6 numbers/sec – for the serotonergic and 4-5 numbers/sec for the noradrenergic stimulations of the nuclei of the amygdala, hypothalamus, midbrain reticular formation, and the medial nuclei of the septum and hippocampus; 2) changes in the amplitudes of the oscillations of synchronized potential compared to the baseline EEG activity, which reached its peak on the background of administration of cholinomimetics, average values under the administration of 5-HT and was below the baseline level under administration of NA. Considering the available data in the literature about the importance of studying brain structures in the regulation of the pituitary-adrenal cortex, one can assume that changes in the excitability of hippocampal neurons are caused by different electrical and neurochemical effects on the investigated structures of the limbic system. In regulation of the pituitary-adreno-cortical system a variety of neurotransmitters (acetylcholine, NA, 5-HT, dopamine, GABA, prostaglandins, etc.) can participate in (Sapronov, 1998).

The data available in the literature indicate that under the influence of large amounts of corticosteroids in the blood, in the hippocampus rhythmic activity with a frequency of 4-6 numbers/sec is recorded, and under the local application of cortisone or hydrocortisone into the hippocampus, the excitability level of the hippocampal pyramidal cells significantly increases and it forms convulsive activity, which, according to the authors, is the evidence of the direct action of corticosteroids on the dendrites of the hippocampal pyramids (Lishshak, Endreci, 1967; Endroczi, 1972). The results suggest that the regulation of hippocampal theta rhythm, as well as the functional activity of the hypothalamic-pituitary system, bears poly-mediatory character and is not determined strictly by a single brain monoaminergic mechanism, ensuring the reliability of the pituitary-adrenal response to these pressures, which is very important in maintaining the homeostasis. All these, obviously, present the huge compensatory potential of the CNS. A complete and irreversible blockade of the hippocampal EEG, induced by destruction of DAP, clearly indicates that under the given conditions the hypothalamic-pituitary system is posed at low level-violation formation rate of secretion of ACTH and corticosteroids. So, the results of the study indicate to the modulating effects of the limbic brain structures on the hippocampal theta rhythm and obviously on the hypothalamic-pituitary system, as well as the activating role of the amygdale in the activity of the hypothalamic neurons. All above said indicate that a prerequisite for the regulation of excitability of the hippocampal neurons is the integrity of the amygdalo-hypothalamic connections, through which the regulatory effect on the activity of the amygdalo-hypothalamic neurosecretory cells is realized.

REFERENCES

Berridge C.W., Espana R.A. (2000) Synergistic sedative effects of nonadrenergic α1 and α2 receptor blockade in forebrain electroencepha-lographic and behavior indices. *J. Neuroscience*, **99(3):** 495- 505.

Endroczi E. (1972) Role of glucococrticoids in controlling pituitary-adrenal function. *Acta med. Acad. Scient. Hung.*, **29(1-2):** 49-59.

Fujimori B., Yokota T., Ishibashi Y., Takei T. (1958) Analysis of the electroencephalogram of children by histoqram method. *EEG and Clin. Neurophysiol.*, **10(2):** 241-252.

Gasanov G.G., Kasimov A.É., Bagirova R.M. (1989) Contributions of the amygdala and hypothalamus to the formation of hippocampal theta activity. *Nauchnye Dokladi Vyss. Shkoly, Biol Nauki*, **№3:** 51–53.

Kichigina V.F., Kutyreva E.V. (2002) Modulation of theta activity in the septo-hippocampal system by2-adrenoreceptor agonist clonidine. *Zh. Higher Nervous Activity*, **52(2):** 195-204.

Kitchigina V.F. (2006) Regulation mechanisms and functional significance of theta oscillations in the septohippocampal system of the brain. *Abstract of dissertation for the degree of Doctor of Biological Sciences*, Moscow, Pushchino.

Kitchigina V., Popova I., Sinelnikova V., Malkov A., Astasheva E, Shubina L., Aliev R. (2013) Disturbances of septohippocampal theta oscillations in the epileptic brain: Reasons and consequences. *Experimental Neurology*, **247:** 314-327.

Lishshak K., Endreci E. (1967) Neuroendocrine regulation of adaptation activity. Budapest: Academy of Sciences of Hungary, 145 p.

Mysin I.E., Kitchigina V.F., Kazanovich Y. (2015) Modeling synchronous theta activity in the medial septum: key role of local communications between different cell populations. *J. Comput. Neurosci.*, **39(1):** 1-16.

Sapronov N.S. (1998) Pharmacology of the hyperphoresis adrenal system. SPb .: Special lit., 336 p.

Smythe J.W., Christie B.R., Colom L.V. et al. (1991) Hippocompal theta field activity and theta-on/theta-off all discharges are controlled by an ascending hypothalamo-septal pathway. *J. Neuroscience*, **11:** 2241-2248

Steriade M. (1996). Arousal: revisiting the reticular activating system. *Science*, **272:** 225-226.

Vertes R.P. (1992). PHA-L analysis of projections from in the supramammillary nucleus in the rat. *J. Comp. Neurology*, **326:** 595-620.

Hippokampal teta ritminin tənzimlənməsində beyin neyrokimyəvi sistemlərinin rolu

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Dovşan üzərində aparılan xroniki təcrübələrdə, dorsal amigdalofugal yolun elektrolitik zədələnməsi, ventraldan fərqli olaraq, hipokampal teta ritminin tam və dönməz blokadasına səbəb olduğu göstərilmişdir. Hippokampda teta ritminin formalaşmasında səbəb olan amigdala, hipotalamus, retikulyar formalasiyasının müxtəlif nüvələrinin və septumun medial nüvəsinin elektrik və kimyəvi qıcıqlanması dorsal amigdalofugal yolun məhv olması şəraitində onlara xas olan xüsusiyyətlərini nümayiş etdirmədi. Hippokampda EEG-nin bərpası yalnız karbokolin və strixninin intrahippokampal enjeksiyası ilə müşahidə edildi. Güman olunur ki, hippokampal neyronların həyəcanlılığının tənzimlənməsinin şərtlərindən biri də amigdala hipotalamik neyrosekretor hüceyrələrin fəaliyyətinə tənzimləyici təsir göstərdiyi dorsal amigdalofugal yolun bütövlüyüdür.

Açar sözlər: *Hipokampal teta ritmi, dorsal və ventral amigdalofugal yollar, elektro-, xemostimulyasiya, dağılma*

Роль нейрохимических систем мозга в регуляции гиппокампального тета-ритма

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В проведенных на кроликах хронических экспериментах было показано, что электролитическое повреждение дорсального амигдалофугального пути, в отличие от вентрального, приводит к полной и необратимой блокаде гиппокампального тета-ритма. Электро- и хемостимуляция различных ядер амигдалы, гипоталамуса, ретикулярной формации и медиального ядра септума, приводящие к возникновению тета-ритма в гиппокампе, не проявляли свойственного им характера разрушения в условиях дорсального амигдалофугального пути. Восстановление ЭЭГ в гиппокампе отмечалось только при внутригиппокампальном введении карбохолина и стрихнина. Предполагается, что одним из условий регуляции возбудимости нейронов гиппокампа является целостность дорсального амигдалофугального пути, посредством которого осуществляется регуляторное влияние амигдалы на деятельность гипоталамических нейросекреторных клеток.

Ключевые слова: *Гиппокампальный тета-ритм, дорсальный и вентральный амигдалофугальные пути, электро-, хемостимуляция, разрушение*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 116-124 (2020)

A study of accumulation and quantity of microelements with high toxic effects on bone and muscle tissue of reptiles in urbanized areas of Absheron

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Accepted for publication: 26 May 2020

The purpose of this research is to determine the quantity of some toxic microelements in bone and muscle tissue of background reptile species, compare this quantity with average standard quantity and thereby to study the impact of environmental pollution on reptiles. The accumulation and amount of microelements with high toxic effects have been studied in bone and muscle tissue of following Reptile species - Water snake (*Natrix tessellata* Laurenti, 1768), Mediterranean turtle (*Testudo graeca* Linnaeus, 1758) and Caspian bent-toed gecko (*Cyrtodactylus caspius* Eichwald, 1831) that have been collected from urbanized areas of Absheron peninsula. Studied microelements are copper Cu, nickel Ni, lead Pb, cadmium Cd and zinc Zn. All the five microelements that we have studied are mostly accumulated in the body of Caspian bent-toed gecko. It should be noted that the Caspian bent toed gecko is very small and more functionally active among background reptile species that we studied. Its occurrence in oil, gas wells and in areas exposed to technogenic and antropogenic pollution, indicates its plasticity and tolerance to toxic microelements.

Keywords: *Technogenic, deionized water, spectrometer, microelement, bone and muscle tissue, biodiversity, urbanization*

INTRODUCTION

As it is known urbanization is the transformation of natural and agricultural areas into towns and cities and as a result the number of urban population increases. At the same time the different fields of industry develops in connection with urbanization. All these factors cause the contamination of soil, pollution of water systems and it can result in health threats to urban fauna (Croteau et al., 2008) According to the literature, macro and microelements are not synthesized in the animal body, so they enter the organism from the external environment - inside food, water, air and take part in the synthesis of high-molecular organic and inorganic substances (Grajdankina, 2001; Chernykh et al., 2004; Baimova et al., 2007; Orbelis et, al., 2008; Bikova, 2014).

doi.org/10.29228/jlsb.49

In recent years, the Absheron peninsula has been exposed to impact of urbanization. Our rese- arch have been done with reptiles collected from urban areas of Absheron peninsula. The strongly urbanized areas of Absheron peninsula differ from other territories with the development of oil and gas, metallurgic and medicinal industries. Due to anthropogenic and technogenic factors, this area is polluted with variety of pollutants, as well as heavy metals. Heavy metals are known to be naturally occurring compounds, but anthropogenic activities introduce them in large quantities in different environmental compartments. This leads to the environment's ability to foster life being reduced as human, animal and plant health become threatened. This occurs due to bioaccumulation in the food chains as a result of the nondegradable state of the heavy metals (Masindi et al., 2017).

The long-term exposure to heavy metals in the environment represents a threat to wild populations, affecting communities and putting ecosystem integrity at risk. Therefore this type of exposure represents a threat to a biodiversity (Tovar-Sanhez et al., 2018). It has been studied that aquatic and terrestrial ecosystems in urban environments often exhibit a loss of sensitive species, loss of species numbers (decrease in diversity) and increases in numbers of pollution-tolerant organisms and it can lead a loss of particular species from the area. (Croteau et al., 2008).

Thus, the studying of the accumulation and quantity of toxic microelements in bone and muscle tissue of reptiles in urbanized territories of Absheron peninsula, becomes more relevant. These animals are directly related to the soil and vegetation, and the probability of transferring the microelements from the soil to plants and from plants to the body of reptiles is high. (Kist, 1987; Khlebnikova et al., 2013). The turtles are feeding on plants, the geckoes mainly on insects and water snakes on aquatic invertebrates. So, heavy metals, may enter the trophic chain through primary producers (plants) and invertebrates that live in the soil and in water (Tovar-Sanhez et al., 2018). Some important anthropogenic sources which significantly contribute to the heavy metal contamination in the environment include automobile exhaust which releases lead Pb; smelting which releases copper Cu and zinc Zn; burning of fossil fuels which release nickel Ni (Masindi et al., 2017).

MATERIALS AND METHODS

The objects of our research are the Mediterranean turtle (*Testudo graeca* Linnaeus, 1758) from the order Turtles (*Testudines*), the water snake (*Natrix tessellata* Laurenti, 1768) from the order Snakes (*Serpentes*) and the Caspian bent-toed gecko (*Cyrtodactylus caspius* Eichwald, 1831) from the order Lizards (*Sauria*). All these orders belong to the class of Reptiles. These species are predominantly exposed to the effects of anthropogenic and technogenic factors during the urbanization process in the Absheron Peninsula. The route method was used for the collection of materials. The amount of the following microelements - nickel, copper, lead, cadmium and zinc, which have toxic effects and are needed for the normal functioning of the body, have been studied in the muscle and bone tissue of the selected reptile species. These microelements have toxic effects when their amount is higher than the norm, and in the case of their deficiency, the functional activities of the body are impaired. However, the proper amount of these microelements is actively involved in regulating the physiological and biochemical functions of the body.

For the biochemical analysis 10 specimens were taken from each of the Caspian bent-toed geckoes and water snakes, as well as 7 specimens from the Mediterranean turtles. It should be noted that, taking into consideration the sharp decline in the number of Mediterranean turtles in recent years and their inclusion in International Union for Conservation of Nature's Red List of Threatened Species, samples of the turtles have been taken from bone and muscle tissue of damaged or dead bodies that were found on the roadside. Furthermore, during the extraction of the material from the Caspian bent-toed gecko, it was sampled from both muscle and bone tissues for the chemical analysis of microelements, because, due to its small quantity it was not well anatomized. Accordingly, the muscle and bone tissue of the water snakes that we investigated were analyzed together. Since both tissues are a major component of the supporting apparatus, it is more advisable to perform this analysis by using both muscle and bone tissue. Bone tissue and somatic muscles (that form a separate tissue group) are important for the body due to their biological and physiological properties. Considering this point, we aimed to study the amount of toxic microelements that have been found in both tissues.

The quantitative analysis of the collected material was carried out at the Institute of Radiation Problems of ANAS in the Laboratory of "Physics and Chemistry of Harmful Impact on the Environment". The analysis was performed using the AAS-Atomic Absorption Spectroscopy method. To collect research animals we have used the routing method. Atomic Absorption Spectrometer 220 FS was used for determining the accumulation and quantity of toxic microelements in the bone and muscle tissue of the animals we investigated. The advantage of Atomic Absorption Spectrometer is that it is possible to identify several elements in the same

solution with high sensitivity, selectivity, and spending little time. The process of analyzing with this device is carried out in 3 stages: (1) Preparation of tubes; (2) Sample preparation; (3) Analysis stage.

Preparation of tubes: Firstly, water is poured into the deionizer (MILLIPORE) and it is removed from ions to obtain deionized water. The tubes are first rinsed with normal water and shampoo and then washed 2-3 times with deionized water. Then the tubes are kept in 10% HNO3 solution for 2 days. After it the acid is removed from them and they are washed 3 times with deionized water. Then the washed tubes are put into a microwave oven (MILESTONE ETHOS PLUS High Performance Microwave Labstation) at 150 0C for 15 minutes for cleaning. After 1 day, the acid is removed from them, they are washed with deionized water 3 times and dried in the drying oven (Oven / Incubator). Pestle and mortar which are used in the homogenizing of samples are cleaned with the above mentioned procedures and dried in a drying oven for 5 minutes.

Sample Preparation: Dried samples are homogenized with the pestle and mortar. The aim here is to increase the touch surface area of the sample and acid in order to reduce the solubility time. The samples are then poured into the microwave's oven of 1g each, then 10 ml 65% HNO3 and 5ml 30% H2O2 are added and kept closed for 1 day. Then the microwave containers are opened, are relieved the pressure, and then are closed again and are placed in a microwave's oven to let all the metal contained transfer to the acid.

Microwave sample containers (that were kept in the acid) should be washed twice with deionized water and dried in a 40^{o}C drying oven for 10 minutes. Samples which are removed from microwave should be diluted to the required concentration for analysis with the AAS device. Deionizing water is used for dilution. The containers used for the dilution are also cleaned according to the above cleaning rules. Then we stick the sample name, number, weight and dilution percentage (DF-dilution factor) on tubes.

Samples are emptied from microwave ovens into centrifugal tubes of 50 ml, the walls of the tubes are washed with deionized water and added to the samples. The volume is then completed to 50 ml with deionized water.

Analysis stage: The next step is to prepare standards for each metal to be determined. The standard solutions used to form a calibration chart when performing element analysis in AAS are prepared by diluting the stock standard of that element. The dilution is made with 0.5% HNO3 solution. The samples are then poured into the tubes after being diluted 10 times, 0.1 ml of each metal stock standard, 2ml HNO3, 1ml of H_2O_2 are added and then deionized water is added in order to complete solution to 100ml. The prepared solutions are put into a spectrometer for determining the amount of microelements.

RESULTS AND DISCUSSION

The issue of microelement accumulation in various tissues of animals found in urbanized areas or areas contaminated by technogenic factors, was not properly studied in Azerbaijan. The Absheron Peninsula is an area of our republic that has been strongly urbanized and has been exposed to technogenic pollution in recent years. This is due to the intensive development of the peninsula's oil and gas industry, the existence of chemical industry, factories and plants, as well as, a strong tendency of living in urban environments among population. All the factors mentioned above, including anthropogenic factors, have a great impact on wild fauna. Wildlife fauna, including reptile species, that are our research objects, feed on plants, invertebrates (Caspian bent-toed geckoes and Mediterranean turtles), and partially primary vertebrates (water snakes). Thus, the microelements that exist in the body of these creatures, pass through the food chain to the body of the reptiles we investigate and accumulate in their different tissues. Since, the reptiles are generally involved in food chain of biocenosis, studying these micronutrients also has a practical importance (Orbelis et al., 2008; Maksimyuk et al., 2015).

As shown in the table, copper is more commonly found in the bone and muscle tissue of the Caspian bent-toed gecko (6.025 mg/kg), approximately half of this amount has been observed in Mediterranean turtle (4.364 mg/kg) and least amount was found in the water snake (1.322 mg/kg).Copper is released into environment during industrial activities such as cement production, also most domestic

water pipes are copper -based, resulting in frequent contact between water and copper sources in urban areas (Croteau et al., 2008).

Copper is an essential micronutrient that performs vital physiological functions in both human and animal organisms. Copper exists inside many vitamins, hormones, enzymes, respiratory pigments and takes part in metabolism and tissue respiration, stimulates normal blood composition and blood-forming function of bone marrow, regulates the amount of blood cholesterol, intensifies the formation of erythrocytes and leukocytes, strengthens the bone tissue. Low dosage leads to reduction of carbohydrates in blood and affects the metabolism of minerals; the deficiency of this microelement causes the reduction of the growth rate, depigmentation of hair, decrease in hemoglobin levels in blood, damages to cardiac muscles, disruption in the structure of the connective tissue and destruction of blood vessels. In animals, the reduction of absorption and usage of iron occurs which is accompanied by diarrhea and weakness, causing anemia (Diorditsa, 2006; Orbelis et al., 2008; Dobryanskaya et al., 2014;) Excess amount of copper in the body causes functional disorders of respiration, reduction of other microelements - zinc, molybdenum and manganese and long-term excess amount of copper leads to intoxication and poisoning of the body (Chernykh et al., 2004; Dobryanskaya et al., 2014; Sheybak, 2014).

As the mentioned in the table information shows, the amount of copper in bone and muscle tissue of Caspian bent-toed gecko is higher than in other objects of research. This is due to its activity and intensive metabolism. The amount of the copper in Caspian bent-toed gecko's bone and muscle tissue (somatic muscle) is higher than the standard average quantity (Kist, 1987). The reason for this is the technogenic contamination of the peninsula and on the other hand, we can say that the gecko is highly adaptive to this microelement. Copper's toxicity average is over 250 mg (Dobryanskaya et al., 2014).

Table 1. The accumulation of microelements with high toxicity, in the bone and muscle tissue of Mediterranean turtle, water snake and Caspian bent-toed gecko (mg / kg).

Species	Microelements				
	Cu	Ni	Pb	Cd	Zn
Mediterranean turtle	4.364	1.122	0.547	0.055	212.4
Water snake	1.322	1.392	0.564	0.059	274.7
Caspian bent-toed gecko	6.025	2.499	5.060	0.103	554.2

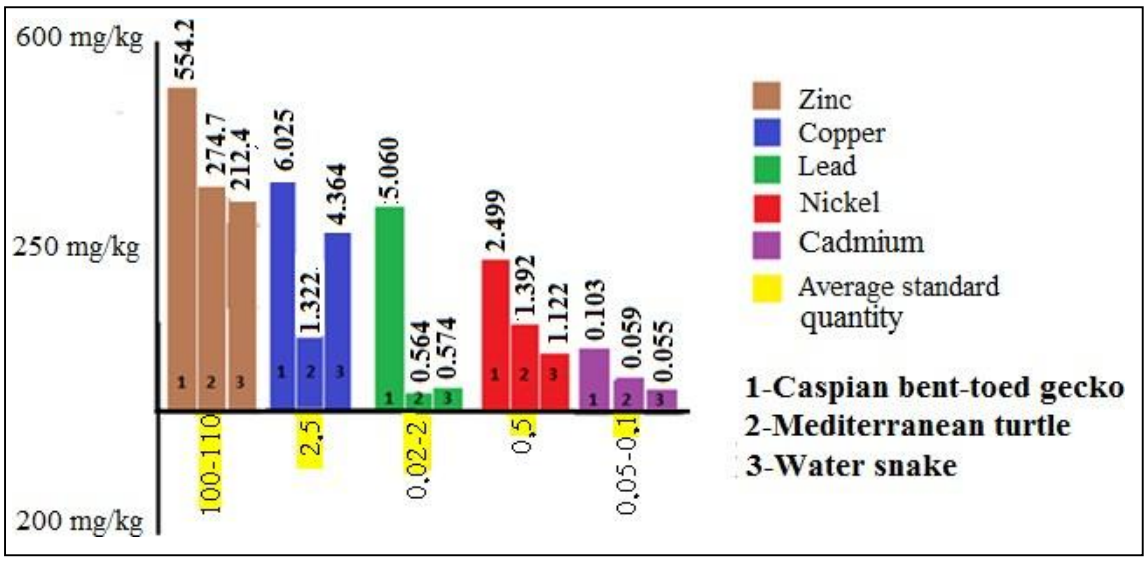

Figure l. The amount of microelements with high toxicity in comparison with the average standard quantity (mg/kg) in the bone and muscle tissue of the Mediterranean turtle, water snake and Caspian bent-toed gecko.

One of the microelements required for the normal development of living organisms is nickel. It is known that the nickel is involved in the fermentation processes in animals and plants. The amount of nickel in the bone and muscle tissues of the reptiles of our research is as follows: 2.499 mg/kg in the Caspian bent-toed gecko, 1.392 mg/kg in the water snake, and 1.122 mg/kg in the Mediterranean turtle. Excess amount of nickel leads to eye diseases (Kornick et al., 2008). This microelement has high allergic properties, so, in 2008 the American Contact Dermatitis Society recognized nickel as "Allergen of the Year" (Diorditsa, 2006). In human and animal organisms, nickel is accumulated in the pancreas and parathyroid gland, accelerates the oxidation of the ascorbic acid and speeds up the passage of sulfhydryl group into the disulfide. As it seems, nickel is one of the most important microelements in the body, which its deficiency and excess amount in the body leads to impairment of physiological processes (Kornick et al., 2008).

Lead is released to the atmosphere from gasoline, as well as, oil combustion, cement and metallurgic industries cause lead accumulation in the soil and atmosphere. Lead is one of the most important microelements for the body (Diorditsa, 2006; Baimova et al., 2007). Its biological value is not sufficiently studied yet, but experiments on laboratory animals show that it participates in the metabolism of bone tissue, stimulates growth, takes part in iron metabolism, affects the hemoglobin concentration and the activity of some enzymes. Lead levels in human body vary from 2 to 200 mg.

Among the reptiles of our research, the accumulation of lead is more in bone and muscle tissue of the Caspian bent-toed gecko 5.060 mg/kg. It should be noted that, the lead is mainly accumulated in bone tissue, liver, kidney and brain (Diorditsa, 2006; Duskayev et al., 2014). In water snake and in Mediterranean turtle small amounts of lead have been observed, respectively 0.564 and 0.547 mg/kg (Figure 1). Different accumulation of lead in the studied animals' bone and muscle tissue depends on their nutrition. Lead is absorbed directly into the body by food and air (used during breathing), while excess amount is excreted by the feces (Duskayev et al., 2014).

Cadmium is one of the microelements with high toxicity. Atmospheric air deposition and tire wear on asphalt are major urban sources of cadmium (Croteau et al., 2008). Its deficiency and excessive levels can lead to a number of complications in the body. In all living organisms cadmium is found to weigh up to 0.5 mg per kg. Despite the fact that it is present in all living things, its physiological significance has not been studied in details, yet. However, scientists have found out that cadmium is involved in the metabolism of some micronutrients, such as zinc, iron, silver and calcium, takes part in sugar metabolism and in activation of certain enzymes in the body. Cadmium is mainly concentrated in the kidneys (30-60% of the total), liver (20-25%), pancreas, long bones, spleen and other tissues and organs. Sublethal effects of cadmium exposure on herpetofauna have also been demonstrated (Croteau et al., 2008).

It should be noted that although cadmium is considered to be one of the most dangerous toxic elements, its application in medicine has yielded positive results. Thus, patients suffering from heart disorders are provided with nickel-cadmium batteries in their chest cavity in order to supply them with mechanical energy. However, excessive accumulation of cadmium in the body may lead to certain diseases. One more interesting fact: In the 50s of the last century in Japan the local population became infected with a disease called "the Italian disease". The disease was a result of the heavy pollution of the environment. It was revealed that, people in the area where the disease was spread were fed on rice and seafood with high cadmium content, or more precisely, these individuals daily consumed about 600 μg of cadmium. The daily safe dose for a person is 1 μg per kg body weight.

The amount of cadmium in bone and muscle tissues of the reptiles that we investigated is as follows: 0.103 mg/kg in the Caspian bent-toed gecko and approximately equal in the water snake and the Mediterranean turtle (respectively 0.059 mg/kg and 0.055 mg/kg). As it seems, the amount of cadmium in muscle and bone tissue of all three reptiles is much lower than the standard average quantity (Kist, 1987).

Zinc is the most common microelement in the body after iron. Sources of zinc in urban environments include tire wear, brake lining wear and corrosion from galvanized steel barriers (Croteau et al., 2008) It is a component of more than 2,700 enzymes, and has a catalytic function in up to 70

enzymes (Khlebnikova et al., 2013; Maksimyuk et al., 2015; Khabarov et al., 2012). Due to its antioxidant properties it takes part in DNA reparation. The bio-physiological significance of zinc microelement has been studied extensively in medicine, agricultural animals and some species of wild fauna (Klug, 2010; Shtikova, 2015; Rebezov et al., 2015). Despite the biological importance of a zinc as a microelement, zinc pollution can negatively impact reptiles (Croteau et al., 2008). Its deficiency and excess amount causes certain disorders in the body, primarily the disruption of normal growth and development. In this regard, interesting information is given in the works of N.N.Maximyuk and M.B.Rebezov (Maksimyuk et al., 2015) about accumulation of heavy metals in wild boar meat. The authors suggest that, it is not advisable to use boar meat as food, because heavy metals including zinc accumulate in the internal organs and bones of this animal. Studies show that the zinc microelement is sufficiently deposited in bone tissue of hunting birds and mammals, so it is clear, that it will accumulate also in muscle tissue, which is directly related to bone tissue (Sheybak, 2014; Sheybak, 2015). The accumulation of eco-toxicants, including zinc microelement in wild fauna species, which live in areas where urbanization and technogenic pollution are widespread, contributes to serious disruption of the ecosystem (Klug, 2010; Rebezov et. al., 2015).

Cu, Ni and Zn are essential, serve as micronutrients and are used for redox processes, to stabilize molecules through electrostatic interactions; as components of various enzymes and regulation of osmotic pressure. Too low concentrations of heavy metals lead to a decrease in metabolic activity and too high concentrations lead to toxicity (Rathoure et al., 2017).

As a result of chemical analysis, it was determined that the copper microelement is in the second place after zinc among studied microelements in reptile species. Thus, in bone and muscle tissue of the Caspian bent-toed gecko the amount of zinc was 554.2 mg/kg, while the amount of copper was 6.025 mg/kg; in water snake the amount of zinc and copper was respectively 274.7 mg/kg and 4.364 mg/kg, in Mediterranean turtle the amount of zinc and copper were respectively 212.4 mg/kg and 1.322mg/kg.

Zinc microelement is found in the tissues of all plant and animal organisms. In the body of an adult human the quantity of zinc is about 2 g and this number is twice less than the amount of iron. It is known that, 20% total amount of zinc is accumulated in the bone, 6% in the blood plasma, 2.8% in the erythrocytes, 3% in the liver, and 65% in the muscle tissue (Sheybak, 2014). The amount of zinc in various food products is as follows: 20-40 mg/kg in beef, pork and mutton, 15-30 mg/kg in fish meat and 60-100 mg/kg in oysters. In chicken eggs the amount of zinc is 15-20mg, in carrot, beet and potato is 10 mg/kg, in bone and muscle tissue is about 100-110 mg/kg (Yanovich, 2014; Shtikova, 2015).

CONCLUSION

As mentioned in discussions amount of microelements, that we studied in bone and muscle tissue of reptiles differs depending on species and microelements. Some of them are below, while others are above the standard average and it is related to disturbance of the ecological balance. First of all, these microelements are excessive in external environment and on the other hand, the amount of some microelements exceeds the norm in muscle and bone tissue of reptiles. So, it helps us to come to conclusion that some of these organisms have a high ability to accumulate microelements and adapt to them. All the five microelements that we have studied are mostly accumulated in the body of Caspian bent-toed gecko. It should be noted that the Caspian bent toed gecko is very small and more functionally active among background reptile species that we studied. Its occurrence in oil, gas wells and in technogenic areas indicates its plasticity and resistance to toxic microelements. Therefore, high levels of microelements detected in the Caspian bent-toed gecko do not cause lethal effects. Toxic microelements in bone and muscle tissue of studied reptiles are higher than the standard average and one of the reasons why they do not have a lethal effect is that, these micronutrients are accumulated in functionally active bone and muscle tissue. The toxic effects of microelements are directly related to which organ of body it is accumulated in and this issue has not been studied in detail, yet (Chornix et. al., 2004).

As shown from our research, reptiles those are found in urbanized areas of Absheron peninsula are exposed to an impact of heavy metals that generate a great threat for their health and survival. Some of them (Caspian bent-toed gecko) can adapt and survive in urban conditions, while others such as Mediterranean turtle are at risk of extinction. The reptiles are an important ring of the trophic chain and changes in their numbers are directly affect the other animals and it cause the disturbance of the ecosystem. Therefore, we should find the solution ways for protection of these animals from contamination with heavy metals and at the same time for conservation of biodiversity. In our future studies we are planning to determine the negative effects of heavy metals on reptile species and work on the issue of how to preserve them from toxicological effects of heavy metals.

ACKNOWLEDGEMENTS

We are very grateful to the Institute of Radiation Problems and personally to the head of the Laboratory of "Physics and Chemistry of harmful impact on Environment", Ph.D., Associate Professor, Dr. Famel Humbatov and professor Maged Ahmadov, Sattar Mammadzade, Abdullah Chhab for their support.

REFERENCES

Baimova S.R., Redkina N.N., Likasova N.A. (2007) Contents of heavy metals in the organs and tissues of animals from Bash-Kirian Transurals .*News of Bashkir State University*, **12(2):** 27-28 (in Russian).

Bikova EA. (2014) Content and degree of accumulation of toxic elements and heavy metals in domestic mouse in Tashkent city. *Actual problems of studying and conservation of animal kingdom in Uzbekistan*. Tashkent: 23-25 **(**In Russian).

Bikova E.A., Gashev S.N. (2014) Properties of microelement accumulation in mammals' organism related to urbanization. *News of Samara Scientific Centre RAS*, **16(1(4)):** 1144-1148 (In Russian).

Chernykh N.A., Bayeva N.I. (2004) Heavy metals and human health. *News of RUDN University, Ecology and safety of vital functions,* **1(10):** 125-134 (in Russian).

Croteau M.C., Hogan N., Gibson J.C., Lean D., Trudeau V.L. (2008) Toxicological threats to amphibians and reptiles in urban environments. *Herpetological Conservation*, **3:** 197–209.

Diorditsa V.A. (2006) About toxic role of heavy metals. Kharkov; http://www.infmed.kharkov.ua/TojMet.htm ((in Russian).

Dobryanskaya A.N., Shvets T.M., Yanovich D.A., Melnik A. (2014) Properties of copper accumulation in ecosystem components of fishing pool. *Scientific news of LNUVMB named after S.Z.Gjitskov*, **16(3 (60)):** 296-300 (in Russian).

Duskayev G.K., Miroshnikov S.A., Sizova E.A. et al., (2014) Impact of heavy metals on animal's organism and environment of habitat (review). *News of Meat Livestock,* **3:** 7-11 (in Russian).

Grajdankina E.I. (2001) Toxic elements and heavy metals in biosubstrates of animals and human in large industrial centers of Tashkent. In: *Actual problems of utilization helpful fossil reserves*. Ташкент, 245-246. (In Russian)

Khabarov A.A., Budko E.V., Lushov K.A. et al., (2012) Zinc: Actuality and characteristics of biosupplements (review). *Electronic scientific journal. Modern problems of science and education,* {Electronic source} http://science-education.ru/ru/article/view?id=6416

Khlebnikova A.N., Petrunin V.V. (2013) Zinc, its biological role and application in dermatology. *News of dermatology and venereology,* **6:** 100-116 (in Russian).

Kist AA. (1987) Phenomenology of biogeo-chemistry and bioinorganic chemistry. Tashkent: FAN, 236 p. **(**in Russian).

Klug A. (2010) The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annu. Rev. Biochem.,* **79:** 213-231.

Kornick R., Zug K.A. (2008) Dermatitis. Nickel Named Contact Allergen of the Year Nickel- WikiZero https://www.wikizero.com > nickel

Maksimyuk N.N., Rebezov M.B. (2015). Study of xenobiotics in the meat of wild boars.*International scientific-research journal Biological sciences*, **2 (7 (38)):** 81-85. **(**In Russian)

Masindi V., Muedi K.L. (2017) Environmental contamination by heavy metals. In: *Heavy Metals*, **Chapter 7:** p. 2-20.

Orbelis D., Xarland B., Skalny A. (2008) Biological role of macro- and microelements in human and animals. St.Petersburg, *Science*, Page 544.

Rathoure A.K., Rathoure G.K. (2017) Heavy metal pollution and its eco-friendly management. In: *Bioremediation Current Research and Application*, **Chapter 2:** 16-42.

Sheybak L.N. (2015) Role and importance of zinc in perinatology. *Grodno State Medical University* (Belarus), **2:** 30-36.

Sheybak V.M. (2014) The biological significance and homeostatic regulation of zinc in mammals. *Journal. Problems of Health and Ecology*, 11-16. **(**In Russian)

Shtikova O.N., Leqonkova T.I., Stepina T.G. et al. (2015) Zinc deficiency as a risk factor of chronic pathology formation in children. *Actual Problems of Medicine* (Grodno), **2:** 323-325.

Towar-Sanchez E., Hernandez-Plata I., Martinez M.S., Valencia-Cuevas L., Galante P.M. (2018) Heavy metal pollution as a biodiversity threat. In: *Heavy Metal Pollution as a Biodiversity Threat. Heavy Metals,* **Chapter 21:** 383-399.

Yanovich N.E., Yanovich D.O. (2014) Role of micronutrients in vital functions of fish. *Scientific* news *of LNUVMB named after S.Z.Gjitskov,* **16(2 (59)):** 345-373(In Russian)

Abşeronun urbanlaşmış ərazilərindəki sürünənlərin sümük və əzələ toxumasında yüksək toksiki təsirli mikroelementlərin toplanması və miqdarının öyrənilməsi

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Tədqiqat işinin məqsədi sürünənlərin sümük və əzələ toxumasında toplanan toksiki mikroelementlərin miqdarının təyin edilməsi, bu miqdarın standart orta miqdarla müqayisəsi və bu yolla da ətraf mühitin çirklənməsinin reptililərə təsirinin öyrənilməsidir. Abşeron yarımadasının urbanlaşmış ərazilərindən toplanmış reptili növlərinin – Suilanı (*Natrix tessellata* Laurenti, 1768), Aralıq dənizi tısbağası (*Testudo graeca* Linnaeus, 1758) və Xəzər nazikbarmaq gekkonunun (*Cyrtodactylus caspius* Eichwald, 1831) sümük və əzələ toxumasında yüksək toksiki təsirə malik olan mikroelementlərin toplanması və miqdarı öyrənilmişdir. Öyrənilmiş mikroelementlər aşağıdakılardır: mis *Cu,* nikel *Ni,* qurğuşun *Pb,* kadmium *Cd* və sink *Zn.* Məlum olmuşdur ki, öyrənilən 5 mikroelementin hər biri ən çox Xəzər nazikbarmaq gekkonunun bədənində toplanmışdır. Qeyd olunmalıdır ki, Xəzər nazikbarmaq gekkonu öyrənilən növlər arasında ən kiçik və funksional cəhətdən ən aktiv növdür. Bu növün neft və qaz quyularının ətrafında, antropogen və texnogen çirklənməyə məruz qalmış ərazilərdə daha çox yayılması onun plastikliyini və toksiki mikrolelementlərə qarşı tolerantlığını göstərir.

Açar sözlər: *Texnogen, deionizə edilmiş su, spektrometr,mikroelement, sümük və əzələ toxuması, biomüxtəliflik, urbanlaşma*

Изучение накопления и количества высокотоксичных микроэлементов в костной и мышечной тканях рептилий на урбанизированных территориях Апшерона

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Цель этого исследования заключалась в изучении влияния загрязнения окружающей среды на устойчивость рептилий, путем определения количества токсичных микроэлементов, накопленных в их костной и мышечной тканях и сравнения этого количества со стандартными средними. Накопление и количество высокотоксичных микроэлементов определялось в костной и мышечной тканях водяной змеи (*Natrix tessellata* Laurenti, 1768), Средиземноморской черепахи (*Testudo graeca* Linnaeus,1758) и Каспийского тонкопалого геккона (*Cyrtodactylus caspius* Eichwald, 1831), выловленных на урбанизированных территориях Апшеронского полуострова. Изученными микроэлементами являлись: медь (Cu), никель (Ni), свинец (Pb), кадмий (Cd) и цинк (Zn). Показано, что наибольшая концентрация каждого из 5 изученных микроэлементов была отмечена в теле Каспийского тонкопалого геккона. Следует отметить, что Каспийский тонкопалый геккон - самый маленький и наиболее функционально активный среди изученных видов. Широкое распространение этого вида вокруг нефтяных и газовых скважин, в районах, подверженных антропогенному и техногенному загрязнению, указывает на его пластичность и устойчивость к токсичным микроэлементам.

Ключевые слова: *Техногенный, деионизированная вода, спектрометр, микроэлемент, костная и мышечная ткани, биоразнообразие, урбанизация*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 125-130 (2020)

Complex diagnostic methods for non-tumoral pathologies of gastro-esophageal junction (mini review)

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Accepted for publication: 10 April 2020

According to the literature, there is a significant increase in esophageal pathologies in developed countries, which reduces the life quality and working abilities of patients. Timely and correct diagnosis of non-tumor abnormalities of the gastro-esophageal junction is one of the main factors influencing the quality of treatment and the recovery of the patients.

Keywords: *Gastroesophageal junction, GERD, hiatal hernia, gastroesophageal reflux*

Increased level of urbanization and life rhythm, poor nutrition, and negative stress on the background of active changes in the socio-economic structure of society have a negative impact on people's health and, consequently, increase the incidence of gastro-intestinal diseases. Despite of the development of modern medicine and the improvement of diagnostic methods, the diagnosis of gastro-intestinal diseases is still topical. According to the literature, there is a significant increase in esophagus pathology in developed countries, which reduces patients' life quality and their ability to work.

Currently, gastro-esophageal reflux disease (GERD) is one of the important problems of modern gastro-enterology. Given the prevalence of GERD and its complications among the able-bodied population, it can be called the epidemic of the 21st century. Among the pathologies of the upper sections of the digestive tract, GERD is found in 40-50% of the adult population. GERD is prevalent in both children and adults (Волчкова и Оспанов, 2011; Hopkins et al., 2015; Можаровский и др., 2017). According to many authors, the clinical signs of the disease are associated with functional disorders of the gastro-intestinal tract (Велигоцкий и Горбулич, 2007; Шишко и Петрулевич, 2015; Шестакович, 2015; Chenxi et al. 2017). According to the World Health Organization's classification, GERD is a chronic recurrent disease that causes reflux of gastric and/or duodenal secret, and is irrespective of the presence of morphological changes in the gastrointestinal tract as a result of impairment of the motor-evacuator function of the gastro-esophageal zone. In the latest recommendation of the international consensus (Montreal Consensus 2006), GERD is a disease that manifests itself with the symptoms and/or complications of the patient as a result of reflux in gastro-intestinal disorders. GERD has been adopted as an independent disease in 1997 (Henval, Belgium) (Мхаммад и Орозбекова, 2017).

Due to the scope of its spreading, GERD is considerably more popular. Despite of numerous epidemiological studies on GERD and its effects, the used questionnaires and the descriptions of reflux are different. Mainly, Mayo, GERD - Q, DIGEST - Q, RDA and country-specific questionnaires were used for studies. The most commonly accepted clinical description of GERD in the world is the presence of at least once a week a complaint of pyrosis and/or regurgitation. However, surveys also use clinical description such as at least twice a week pyrosis and/or regurgitation, and at least once a year, pyrosis and/or regurgitation. So, it is very difficult to summarize all studies and draw conclusions. At the same time, epidemiological analyzes show that the incidence of GERD varies in eastern and western countries,

doi.org/10.29228/jlsb.50

and even in different geographical areas of the country, different ethnic groups, and people of different races. The most extensive, randomized study of eastern countries was conducted in China in the 1990s by the Mayo survey and it found that 2.5% of the population was suffering from pyrosis and regurgitation, which is considered a complaint of GERD. Subsequent literature data indicate that this figure rose up to 6.2% in China. In Japan, the incidence of GERD occurrence is 16.5%, which is the highest in the Far East. In the Indian Peninsula this figure is 5.3-7.1%. In the European and American continents, the highest values were recorded in the United States (26.2%), Norway (26%) and Sweden (25.9%). A study, conducted in the United States, was also analyzed in epidemiological aspect among subgroups and it was found for 38% of Hispanic people, 14.7% in Asian subgroups, 29.9% in whites, and 22.1% in blacks. As for Turkey, this figure was reflected in studies, as 23%, and the highest was in Russia - 33%. On a whole, gastro-intestinal reflux symptoms in Western Europe and North America are encountered in about 20% of the population and 5% in Asia. Most likely, the actual values of its incidence are higher, as some of the epidemiological groups are asymptomatic patients and atypical forms of GERD. The complications of GERD (esophagitis, Barrett esophagitis and related adrenocarcinoma of the esophagus) are more commonly encountered in western countries (Мхаммад и Орозбекова, 2017; Serhat ve Yüksel, 2017; Akyüz and Soyer, 2017).

The diaphragmatic hernia (hiatal hernia) is one of the main causes of formation GERD. The hiatal hernia (HH) ranks a position in the structure of the digestive tract diseases after gastric ulcer and cholecystitis (Левин и др., 2013; Дронова и др. 2016; Затевахина и др., 2016; Akyüz and Soyer, 2017).

The brightest and the most common complication of the HH is a pain (45-80%). It can irradiate to the neck, ears, shoulders and back. In 10–11% of patients it can simulate angina by irradiating to the back of the breast. According to various literature data, discomfort and aching syndrome are reported in up to 35% of cases in xiphoid process, 25% in the sternum projection and 20% in the heart region. The cause of pain is the compression of the vascular and nerve endings as a result of migration of the cardiac and fundal part of the stomach from diaphragm (Левин и др., 2013; Затевахина и др., 2016; Akyüz and Soyer, 2017).

The diaphragmatic hernia is sub-divided into 4 types: sliding hernia, paraesophageal hernia, mixed hernia and giant hernia. Sliding hernia (type 1) is the most common type (90-95%) and is mainly observed in association with GERD. In sliding hernia (type 1) the gastro-esophageal junction (GEJ) is monitored in the posterior mediastinum with the cardiac proximal. In Type 1 hernias the natural barrier against GER is disrupted, the diaphragm legs pressure on the low esophageal sphincter is reduced, and the proximal cardia displacement causes the opening of Hiss angle, which makes the reflux more susceptible. For Type 2 hiatal hernia while GEJ and cardia remains at the typical location, the gastric fundus or part of the large curvature passes from the diaphragm to the thoracic cavity. Type 3 incorporates the symptoms of both types, no matter how far esophagus and cardia are displaced to the thoracic cavity, and the gastric fundus and the greater curvature are always above. For Type IV other abdominal organs also (spleen, small intestine and colon) are displaced to the thoracic cavity besides stomach and GEJ. (Калинина и др., 2014; Зябрева и Джулай, 2015; Pawluszewicz et al., 2018)

Non-tumoral pathologies of the GEJ are difficult to identify and can easily be ignored and misdiagnosed. Timely and correct diagnosis of non-tumoral pathology of the GEJ is one of the main factors affecting the quality of treatment and the health of patients.

The most reliable method used for the diagnosis of gastro-esophageal reflux is the daily pH measuring of the esophagus.

Esophago-myometry provides information on the myomotoricity of the esophagus. This method is considered a "gold standard" in determining of the motor discoordination of the esophagus. However, the method is less effective in the diagnosis of joint abnormalities of the abdominal cavity.

Another method used in the diagnosis of esophageal diseases is endoscopy. Despite of its invasive nature and creating discomfort in the patient, the method is widely used because of its higher sensitivity than X-ray method and is considered a "gold standard."

The main visualization methods used in diagnostics of the esophagus pathologies are radiology, computed tomography (CT) and endoscopic ultrasound (EUS) techniques. Although all methods are highly informative and play an important role in revealing pathologies of the esophagus, each method has different capabilities, accuracy and sensitivity to the pathology. It should be noted that non-invasiveness of the method plays a major role for the selection of examination methods. As the devices improve, new opportunities are open for diagnostic methods, which change the method's priority in the diagnosis of pathologies.

The most traditional and widely used method in diagnostics of diseases of the esophagus is radiology. The method has sufficient diagnostic capabilities and is 71% informative in the diagnosis of HH. The method allows determining the presence, size, shape of the hernia, functions of the GEJ and possible complications (Туранов и др., 2010; Зябрева и Джулай, 2015; Дронова и др., 2016).

In the last decade, multi-spiral computerized tomography (CT) has been used extensively in the differential diagnosis of GEJ pathology. The main advantage of the CT over the routine radiological and endoscopic techniques is that it allows the GEJ to objectively assess not only the anatomical area but also the diaphragm legs, diaphragm, and other structures of the diaphragm. The American Association of Abdominal Surgeons recommends computerized tomography (CT) of the thoracic cavity organs for the diagnosis of HH. Multi-spiral CT and 3D reconstructive capabilities with peroral contrast increase the sensitivity of the method for the diagnosis of HH (Bilgi ve Batırel, 2013; Журбенко и др., 2015; Семенякина и др., 2017)

Although the endoscopic ultrasound (EUS) provides a detailed overview of the GEJ wall, the ability to perform biopsies under its control raises the importance of the method, the method's dependence on qualification of the person performing the procedure, semi-invasiveness and sedation, as well as lack of equipment in most hospitals, limits the use of the method.

Technological development of ultrasonic devices, lack of ionizing properties of sound waves, easy implementation of the method and non-invasiveness have contributed to the widespread application of ultrasound examination in clinical practice. Transabdominal Ultrasonography (TUS) is the primary method for examination of the abdominal cavity and the peritoneal cavity and usually identifies the next stages of diagnostic search. Although in the pathology of the digestive tract sonography is mainly used for the diagnosis of parenchymal organs, for a long time it has been considered as an obstacle for sound waves due to air in the gastrointestinal tract and the sonographic examination has not been practically performed.

The first information of transabdominal ultrasound of GEJ in children was published by Westra in 1990 (Westra et al., 1990). Later, in 1994 Aliotta and colleagues conducted sonographic analysis of the GEJ in adults (Aliotta et al., 1995). Based on comparative analysis of patients with 18 healthy and 12 patients with HH, author reported that, healthy individuals had a clear visualization of GEJ and abdominal esophageal diameter up to 10 mm, other patients with HH had 16-21 mm diameter and non-clear visualization of GEJ. It was found that precision in measuring diameter values is 90% and non-clear visualization of GEJ is 94.7%. Barone et al. (2006) with the help of TUS in 168 patients measured the diaphragm diameter of the esophagus and compared with endoscopy: in 24 of 29 patients with a diameter greater than or equal to 18 mm, HH was endoscopically confirmed. Д.А.Балагански и др. (2011) reported an increase in diameter of the abdominal portion of the esophagus–75.9%, wall thickness 82.8%, adverse extension 44.8%, The angle of 90 degrees and more in 27.6%, and reflux during examination in 27.6% of children with GERD.

From the literature review it gets clear that the opportunities of TUS are mostly restricted to the diagnosis of gastro-esophageal reflux in newborns, and sometimes in carcinomas, leiomyom and achalasia in adults. The role of TUS in the diagnostic algorithm and its effectiveness in the dynamics of pathological processes has not been fully described. We did not find any literature on TUS addressing the after fundoplication surgery cases. Ultrasound is a method that provides sufficient information about the abdominal cavity and we believe that it can also provide an irreplaceable information on the morphology of the GEJ.

Given the above, it appears that there is a need for the creation of a new diagnostic algorithm

that is more rational, shortens the examination period and allows for the selection of the most optimal surgical method based on the results obtained with the complex application of radiologic

REFERENCES

Ахматов А.М., Тарабаев И.С., Василевский Д.И. (2018) История развития хируругии грыж пищеводного отверстия диафрагмы. *Педиатрия,* **dып. 3:** 77-79.

Балаганский Д.А., Кошель А.П., Врублевская М.Л. (2011) Возможности трансабдоминальной ультрасонографии в диагностике гастроэзофагеальной рефлюксной болезни у детей. *Бюллетень Сибирской Медицины*, **№3:** 111-115.

Велигоцкий И.И.. Горбулич А.В.(2007) Патофизтологические аспекты функциональных расстройств пищеводно-желудочного перехода. *Международный Медицинский Журнал,* **№1:** 71-79.

Волчкова И.С., Оспанов О.Б. (2011) Хирургические аспекты лечения ГЭРБ: показания к операции и результаты применения лапароскопических фундопликаций различного типа. *Медицинская наука и образование Урала***, 12(4):** 126-128.

Дронова О.Б., Третьяков А.А., Каган И.И. и др. (2016) Гастроэзофагеальная рефлюксная болезнь: Вопросы патогенеза и диагностики. *Оренбургскийй Медицинский Вестник,* **IV(4):** 57-62.

Журбенко Г.А., Карпицкий А.С., Панько С.В., Шестюк А.М. (2015) Компьютерная томография органов грудной клетки у пациентов со скользящей грыжей пищеводного отверстия диафрагмы. *Грудная и Сердечно-Сосудистая Хирургия,* **57(1):** 36-42.

Затевахина И.И., Кириенко А.И., Кубышкина В.А. (2016) Абдоминальная хирургия. Национальная руководств: краткое издание. М.: ГЭОТАР –Медиа, 912 с.

Зябрева И.А., Джулай Т.Е. (2015) Грыжи пищеводного отверстия диафрагмы: спорные, нерешенные и перспективные аспекты проблемы (обзор литературы). *Верхневолжский Медицинский Журнал,* **14(вып. 4):** 24-28.

Калинина Е.А., Пряхин А.Н.(2014) Технические аспекты лапароскопической пластики грыж пищеводного отверстия диафрагмы: обзор литературы и собственный опыт. *Вестник Южно-Уральского ГУ. Серия «Образование, здравоохранение, физичекая культура»*, **14(3):** 54-60.

Левин М.Д., Коршун З., Мендельсон Г. (2013) Патологическая физиология гастроэзо-фагеальной рефлюксной болезни. Гипотеза (обзор литературы). *Экспериментальная и Клиническая Гастроэнтерология*, **№5:** 72-88.

Левин М.Д., Мендельсон Г. (2015) Кольцо Щацкого как симптом гастроэзофагеальной рефлюксной болезни. *Вестник Рентгенологии и Радиологии*, **№1:** 5-15.

Можаровский В.В., Цыганов А.А., Можаровский К.В., Тарасов А.А. (2017) Оценка эффективности хирургического лечения гастроэзофагеальной рефлюксной болезни в сочетании с грыжей пищеводного отверстия диафрагмы. *Хирургия***, 6:** с.28-32.

Мхаммад М.М.. Орозбекова Б.Т. (2017) Гастроэзофагеальная рефлюксная болезнь и особенности эпидемиологии (литературный обзор). *Тенденции развития науки и образования*, **№30-1:** 34-40.

Семенякина И.В., Дибиров М.Д., Ерин С.А. и др. (2017) Клинико-инструментальные особенности диагностики грыж пищеводного отверстия диафрагмы. *Московский Хирургический Журнал,* **№2:** 30-37.

Туранов Н.Л., Ламекина Я.А., Зинченко С.А. и др. (2010) Рентгенодиагностика гастроэзофагеальной рефлюксной болезни и ее осложнений. *Вестник Санкт-Петербургского Университета*, **вып. 2:** 195-207.

Шестакович Е.Н. (2015) Вариантная анатомия брюшной части пищевода взрослого человека современными методами исследования. *International Scientific and Practical conference "World Science",* **2(3):** 32-34.

Шишко В.И., Петрулевич Ю.Я. (2015) Гастроэзофагеальная рефлюксная болезень: анатомо-физиологические особенности пищевода, факторы риска и механизмы развития (обзор литературы, часть 1). *Журнал Гродненского Медицинского Университета*, **№ 1:** 19-25.

Akyüz F., Soyer Ö.M. (2017) Gastroözefageal reflü hastalığı nasıl sınıflandırılır? *Türk J.Gastroenteroloji*, **28 (suppl 1):** 10-11.

Aliotta A., Rapaccini G.L., Pompili M. et al. (1995) Ultrasonographic signs of sliding gastric hiatal hernia and their prospective evaluation. *Journal of Ultrasound in Medicine*, **14(6):** 457-461.

Barone M., Dilernia P., Carbonara M. et al. (2006) Sliding gastric hiatal hernia diagnosis by transabdominal ultrasonography: an easy, reliable and non-invasive procedure. *Scand. J. Gastroenterol.*, **41(7):** 851-855.

Chenxi X., Yuwen L., Zhang N. et al.(2017) Gastroesophageal flap valve reflected EGJ morphology and correlated to acid reflux. *BMC Gastroenterology*, **17:** 118.

Hopkins J., Switzer N.J., Karmali S. (2015) Update on novel endoscopic therapies to treat gastroesophageal reflux disease: A review. *World J. Gastrointest. Endosc*., **7(11):** 1039-1044.

Pawluszewicz P., Wojciak P., Diemieszczyk I. et al. (2018)Hiatal hernia – epidemiology, pathogenesis, diagnostic. *Post. N. Med.*, **XXXI (5):** 274-278.

Serhat B., Yüksel E.S. (2017) Gastroözofageal reflü hastalığı prevalansı, insidansı vekomplikasyonlarının (striktür/özofajit/Bar-rett/karsinom) sıklığı dünyanın değişik coğrafi bölgeleriyle karşılaştırıldığında nasıldır? *Turk. J. Gastroenterol.*, **28 (Suppl 1):** S4-S9

Westra S.J., Wolf B.H., Staalman C.R. (1990) Ultrasound diagnosis of gastroesophageal reflux and hiatal hernia in infants and young children. J.Clinic ultrasound, **V.18(6),** p.477-485.

Bilgi Z., Batırel H.F. (2013) Hiatal herniler. *Toraks Cerrahisi Bülteni,* **№2:** 253-258.

Qida borusu - mədə keçidinin qeyri-şiş patologiyalarının kompleks müayinə Metodları

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Ədəbiyyat məlumatlarına əsasən inkişaf etmiş ölkələrdə qida borusu patologiyalarının nəzərəçarpacaq dərəcədə artması müşahidə edilir ki, bu da xəstələrin həyat keyfiyyətini və əmək qabiliyyətini aşağı salır. Qida borusu-mədə keçidinin qeyri-şiş patologiyalarının vaxtında və düzgün diaqnostikası müalicənin keyfiyyətinin artmasına, xəstələrin sağalmasına təsir edən əsas amillərdəndir.

Açar sözlər: *Qida borusu-mədə keçidi, QERX, hiatal yırtıqlar, qastroezofageal reflyuks*

Комплексные методы обследования неопухолевых патологий пищеводно-желудочного перехода

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Согласно литературным данным в развитых странах наблюдается значительное увлечение патологии пищевода, что снижает качество жизни и трудоспособность пациентов. Своевременная и правильная диагностика неопухолевых патологий пищеводно-желудочного перехода является одним из основных факторов, влияющих на качество лечения и раннее выздоровление пациентов.

Ключевые слова: *Пищеводно-желудочный переход, ГЕРБ, хиатальная грыжа, гастро-эзофагеальный рефлюкс*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 131-139 (2020)

Studies of underlying molecular mechanisms of retinitis pigmentosa in experimental model and clinics

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Accepted for publication: 12 June 2020

The article concerns the analysis of underlying molecular mechanisms of retinitis pigmentosa on the experimental model of the rabbits and on the patients diagnosed with this pathology. The studies were conducted on Chinchilla male rabbits having body mass of 2.2-2.6 kg. Serum was obtained from the patients with retinitis pigmentosa. Retinitis pigmentosa was modelled in the rabbits through i.v. administration of monoiodacetic acid (MIAA; grave degree, 26 mg/kg of body mass). With the application of indirect ELISA-test the levels of serotonin-modulating anticonsolidation protein (SMAP; Mekhtiev, 2000) in the hypothalamus, heat shock protein 70 kDa (HSP70) and rhodopsin in the retina and natural anti-SMAP autoantibodies in the serum of the patients were measured. The data were analysed on Student's t-criterion. Significant downregulation of rhodopsin ($p<0.001$) and upregulation of HSP70 ($p<0.001$) in the retina, as well as upregulation of SMAP ($p<0.01$) in the hypothalamus of the MIAA-administered rabbits were noticed. Intra-vitreal administration of SMAP to the MIAA-administered rabbits resulted in a significant upregulation of rhodopsin ($p<0.001$) and HSP70 ($p<0.001$) in the retina. Noticeable downregulation of the titres of natural autoimmune anti-SMAP antibodies in the serum of the diagnosed patients with retinitis pigmentosa relatively to healthy persons of the same age ($p<0.01$) was revealed. Molecular mechanisms underlying hypothalamic trophic regulatory effects on retina receptor cells through retrograde and anterograde axonal transports are considered.

Keywords: *Retinitis pigmentosa, rabbits, serotonin-modulating anticonsolidation protein (SMAP), rhodopsin, heat-shock protein 70, anti-SMAP antibodies, retina, hypothalamus, natural anti-SMAP autoantibodies.*

INTRODUCTION

Retinitis pigmentosa is a severe and to-date incurable form of ophthalmological pathology, manifesting by a damage of receptor apparatus of the retina along with loss of visual function. Although most of researchers relate etiology of retinitis pigmentosa to inborn mutations of the receptor cells (Diager et al., 2013; Xiao et al., 2019), others relate it to disturbances of normal interaction of hypothalamus with the retina and to decline of hypohalamic trophic support of the cellular elements of the retina (Katsnelson, 1958). This idea was partially proven by the studies of Prof. Gadjieva with her colleagues on a model of retinitis pigmentosa on the rabbits, wherein pulse stimulation of ventromedial nucleus of hypothalamus promoted quick recovery of the amplitudes of electroretinogram (ERG) (Agayev et al., 2004). The results mentioned indicate an existence of a significant trophic influence of the hypothalamic nuclei onto maintenance of the retina functions. Earlier in the Academician Abdulla Garayev Institute of Physiology, NAS of Azerbaijan serotonin-modulating anticonsolidation protein (SMAP), being in linear relation with serotonin and realizing its functions on subcellular level, was purified from the rat brain (Mekhtiev, 2000). On the vertebrates it was shown that SMAP possesses anti-mutagenic and anti-toxic activities in response to adverse factors of chemical and bacteriological origin (Allahverdiyeva et al., 2019). Proceeding from the above said, the goal of

doi.org/10.29228/jlsb.51

the present study was analysis of molecular pathogenetic and reparatory mechanisms of the retina and hypothalamus under conditions of experimental dystrophy of receptor apparatus of the retina on the rabbits, as well as on the patients diagnosed of retinitis pigmentosa.

MATERIALS AND METHODS

Biochemical methods. SMAP was purified from the cow brains. The main stages of purification were: (1) precipitation of proteins from the brain extract in 40% ammonium sulphate; (2) gel-chromatography on the column of Sephadex G-150. Process of picking up the immune positive protein fractions after each stage was conducted under control of indirect ELISA-test (Catty, 1989; Mekhtiev, 2000).

In order to pursue changes of retinitis pigmentosa under the studied preparations, the method of measurement of rhodopsin in the retina by indirect ELISA-test was elaborated. Retinas were removed surgically from 35 cow eyes, water-soluble proteins were extracted and 1.8 mg of rhodopsin were purified through centrifugation in sucrose density gradient (Ohguro et al., 1995) and used for production of anti-rhodopsin immunoglobulins. All operations on purification of rhodopsin were realized in the darkened room, elucidated by lantern (25 W) with red filter.

Anti-SMAP and anti-rhodopsin immunoglobulins were produced through 5-6-month immunezation of the rabbits by sub-cutaneous administration of 300 μg of the purified correspondent protein per animal, in mixture with complete Freund adjuvant (Sigma, Germany).

Measurements of the levels of rhodopsin and HSP70 in the retina and SMAP – in hypothalamus of the rabbits of the intact, control and experimental groups were carried out by indirect ELISA-test (Catty, 1989) on polysterene plates (Sigma, Germany). The animals had been anesthetized and sacrificed, and the retinas and hypothalamus were removed at the end of experiments and frozen under a temperature of -70°C. Prior to the beginning ELISA-test, the water-soluble proteins were extracted from the studied samples. The results of the reaction were registered in the photometer for the ELISA-test "Spectra Max 250" (Molecular Devices Co., USA) on the wavelength 492 nm.

Anti-SMAP polyclonal antibodies were purified from the solution of anti-SMAP immunoglobulins through a technique of immune-affinity chromategraphy performed on the column of CNBr-Sepharose (Sigma, Germany) with covalently immobilized SMAP (Osterman, 1985). In a single cycle up to 12 mg antibodies were eluted from the affinity column.

Physiological methods. The studies were undertaken on Chinchilla male rabbits of 2.2-2.6 kg body mass kept in the vivarium conditions. All experiments on administration of the preparations to the animals were conducted in daytime, between 13.00 h and 16.00 h. Retinitis pigmentosa was formed through single administration of MIAA in 2 ml of a sterile saline into the ear edge vein of the anesthetized rabbits at a dose of 26 mg/kg of body mass (grave degree) with needles of size 21 during 3 min (Agayev et al., 2004). Retina was removed from the rabbit's eyes in the darkened room, illuminated with lantern (25 W) with red filter.

As corpus vitreous of the eye is poorly washed with biological liquids and does not have blood supply, excluding spreading preparations from one eye into another one, the studies were realized on both eyes.

The main series of the studies were conducted over the following scheme. In the 1st series of studies the rabbits (n=4) were i.v. administered with MIAA and after 12 days the anaesthetized animals were sacrificed and the retinas from the both eyes (8 eyes) and hypothalamus were removed and water-soluble proteins were extracted; with application of the indirect ELISA-test in the retina the levels of rhodopsin and HSP70, while in hypothalamus the level of SMAP was measured.

In the 2nd series of studies 3 groups of animals were formed: 1) intact group (n=4; 8 eyes); 2) control group (n=4; 8 eyes) and 3) experimental group (n=4; 8 eyes). In the control group of animals MIAA was i.v. administered and after 22 days they were anaesthetized and sacrificed and retina was removed. In the animals of the experimental group retinitis pigmentosa was formed and after 15 days they were administered with 150 μL of SMAP at a concentration of 1.5 mg/mL in the sterile saline into the corpus vitreous of both eyes through the pars plana during 2 min. 7 days from administration of SMAP (22nd day after administration of MIAA) the anaesthetized rabbits were

sacrificed, the retinas were removed from both eyes, water-soluble proteins were extracted and the level of HSP70 was determined in the retina of the rabbits of all groups.

In the 3rd series 3 groups of animals were formed: 1) intact group (n=4; 8 eyes); 2) control group (n=4; 8 eyes) – i.v. administration of MIAA plus intravitreal administration of inactivated SMAP; and 3) experimental group (n=4; 8 eyes) – i.v. administration of MIAA plus intravitreal administration of SMAP. The preparations were administered in an amount of 150 μL at a concentration 1.5 mg/mL in a sterile saline in 2 min on the 5th day after administration of MIAA and after 7 days the anaesthetized animals were sacrificed and levels of rhodopsin and HSP were evaluated in the protein extract of the retina.

In the 4th series of studies 3 groups of animals were formed: 1) 1st control group (n=4; 8 eyes); 2) 2nd control group (n=4; 8 eyes); 3) experimental group (n=4; 8 eyes). The animals of both control, as well as experimental groups were i.v. administered with MIAA and after 15 days the rabbit non-immune γ-globulins or anti-SMAP polyclonal antibodies were administered into the corpus vitreous of the animals of the 2nd control and experimental groups, correspondently. The preparations were administered in an amount of 200 μL at a concentration of 1.8 mg/mL, in a sterile saline, slowly, during 2 min. 7 days later, the rabbits were sacrificed, the retinas were removed from the both eyes, water-soluble proteins were extracted and the levels of rhodopsin and HSP70 were determined.

In the 5th series of studies the rabbits (n=3) were immunized with SMAP as described above for 3 months. Blood samples were taken from the rabbits and the level of anti-SMAP immunoglobulins was evaluated by the indirect ELISA-test. The anaesthetized animals were sacrificed, eyes were removed, water-soluble proteins were extracted and the levels of HSP70 and rhodopsin were measured in the retina.

In the 6th series of studies in the observed patients retinitis pigmentosa was diagnosed in the clinics of Academician Zarifa Aliyeva National Center of Ophthalmology by recording ERG in response to light flashes presented to the patients' eyes with a frequency of 0.2 Hz, duration of 5 sec and duration of a single stimulus 200 msec. From patients (n=9) and healthy volunteers (n=9) blood samples were taken from the vein, serum was saved, diluted 100 times and used as the first antibodies in the indirect ELISA-test in order to determine the level of natural anti-SMAP antibodies (Poletayev, 1995).

The averages of the levels of the studied antigens in the hypothalamus and retina of both eyes of the rabbits as well as the levels of natural anti-SMAP autoantibodies in the serum of patients were calculated within each group and analyzed on Student's t-criterion.

The work is complied with the ARVO statement on the use of animals in scientific research.

RESULTS

In the 1st series of studies the animals were administered with MIAA and 12 days later sacrificed and retinas from both eyes and hypothalamus were removed. Evaluation of the level of rhodopsin and HSP70 in the retina revealed noticeable downregulation of rhodopsin and upregulation of HSP70. In particular, if the level of rhodopsin in the intact animals (n=4; 8 eyes) made 0.275±0.011 optic units, in the animals of the experimental group (n=4; 8 eyes) its level was 0.207±0.007 optic units ($p<0.001$; Fig. 1). In this case, the level of HSP70 in the retina of the intact animals made 0.094±0.004 optic units, while in the animals of the experimental group its level made 0.14±0.007 optic units ($p<0.001$; Fig. 1). Moreover, upregulation of SMAP was revealed in the hypothalamus in the animals of the experimental groups. Particularly, the level of SMAP in the animals with retinitis pigmentosa made 0.298±0.009 optic units, whereas in the intact animals its level was 0.24±0.01 optic units ($p<0.01$; Fig. 1).

In the 2nd series of studies for the purpose of clarifying, if SMAP actually upregulates synthesis of HSP70, the study of the effect of SMAP on the level of HSP70 in the retina of the rabbits with this pathology was undertaken. 7 days after intravitreal administration of SMAP (150 μL, 1.5 mg/ml, in 2 min) sharp upregulation (23 times) of HSP70 in the animals of the experimental group relatively to the control animals (received MIAA) was revealed. Particularly, if the level of HSP70 in the retina of the intact animals (n=4; 8 eyes)

made 0.367±0.04 optic units, in the control animals (n=4; 8 eyes) – 0,039±0.001 optic units (p<0.001), in the animals of the experimental group (n=4; 8 eyes) its level made 0.902±0.042 optic units (p<0.001; Fig. 2).

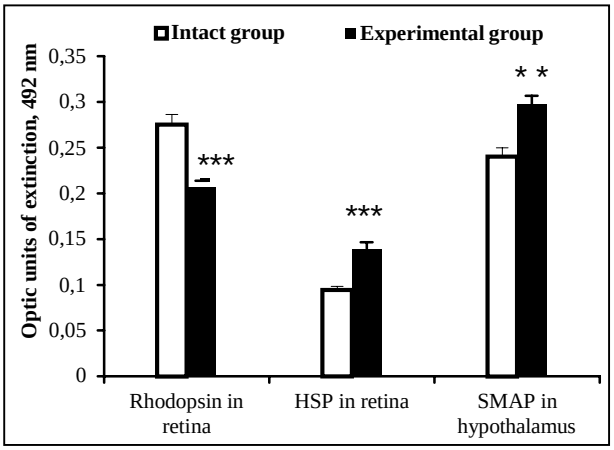

Figure 1. Effects of i.v. administration of MIAA on the levels of rhodopsin and HSP70 in the retina, and on the level of SMAP in the hypothalamus of the rabbits (n=4, 8 eyes).* - p<0.01; *** - p<0.001.

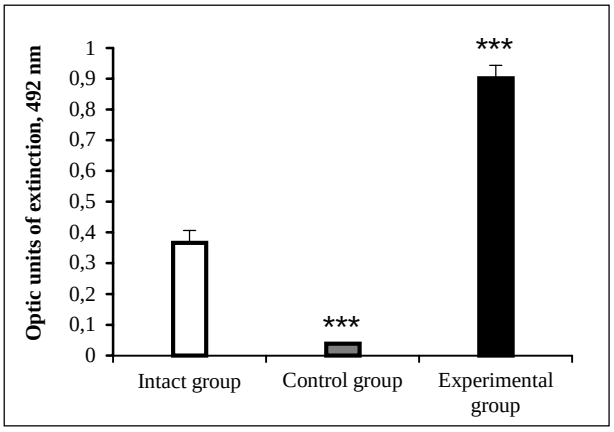

Figure 2. Effect of intra-vitreal administration of SMAP 15 days after i.v. administration of MIAA on the level of HSP70 in the retinas of the rabbits (n=4, 8 eyes). *** - p<0.001.

Hence, if under MIAA the level of HSP70 in the retina downregulated drastically, then under administration of SMAP the level of HSP70 on the animals with retinitis pigmetosa not only overcame the controls, but significantly exceeded the level of the intact animals (p<0.001).

In the 3rd series of studies on 5th day after i.v. administration of MIAA the animals were administered intra-vitreally with active SMAP or heat-inactivated SMAP. After 7 days since administration of SMAP (150 μL, 1.5 mg/mL, in 2 min) noticeable upregulation of rhodopsin in the retina was revealed. In particular, in the intact animals (n=4; 8 eyes) the level of rhodopsin in the retina made 0.187±0.005 optic units, in the animals of the control group (n=4; 8 eyes; inactivated SMAP) - 0.13±0.008 optic units (p<0.001), while in the animals of the experimental group (SMAP; n=4; 8 eyes) – 0,193±0.011 optic units (p<0.001; Fig. 3A). Moreover, the level of HSP70 in the retinas of the intact animals made 0.085±0.004 optic units, while in the control animals – 0.102±0.004 optic units (p<0.05), and in the animals of the experimental group – 0.123±0.005 optic units (p<0.001; Fig. 3B).

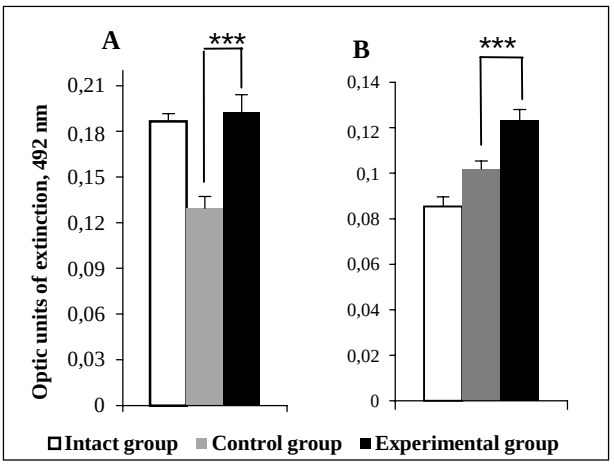

Figure 3. (A) Effect of intra-vitreal administration of SMAP 5 days after i.v. administration of MIAA on the level of rhodopsin in the retinas of the rabbits (n=4, 8 eyes). ***- p<0.001. **(B)** Effect of intra-vitreal administration of SMAP 5 days after i.v. administration of MIAA on the level of HSP70 in the retinas of the rabbits (n=4, 8 eyes). * - p<0.05; *** - p<0.001.

Thus, under intra-vitreal administration of SMAP on the 5th day after injection of MIAA group simultaneous upregulations of rhodopsin and HSP70 in the retinas of the animals of the experimental were noted.

The goal of the 4th series of studies included analysis of the effect of polyclonal antibodies-mediated blockade of SMAP on the level of rhodopsin in the retina of the rabbits with prior induced

retinitis pigmentosa. It was found that intra-vitreal administration of the anti-SMAP antibodies (200 μL, 1.8 mg/mL, during 2 min) to the rabbits with retinitis pigmentosa resulted in considerable upregulation of the level of rhodopsin and HSP70 relatively to their values in the animals of the 1st (MIAA) and 2nd (MIAA plus non-immune γ-globulins) control groups. Particularly, in the animals of the 1st control group (n=4; 8 eyes) the level of rhodopsin made 0.182±0.012 optic units, in the 2nd control group (n=4; 8 eyes) – 0.184±0.009 optic units, whereas in the animals of the experimental group (n=4; 8 eyes) its level was 0.242±0.012 optic units ($p<0.01$ on Student's t-criterion; Fig. 4). Correspondently, in the animals of the 1st control group the level of HSP70 made 0.082±0.012 optic units, in the 2nd control group – 0.109±0.004 optic units, while in the animals of the experimental group its level was 0.158±0.01 optic units ($p<0.001$; Fig. 5).

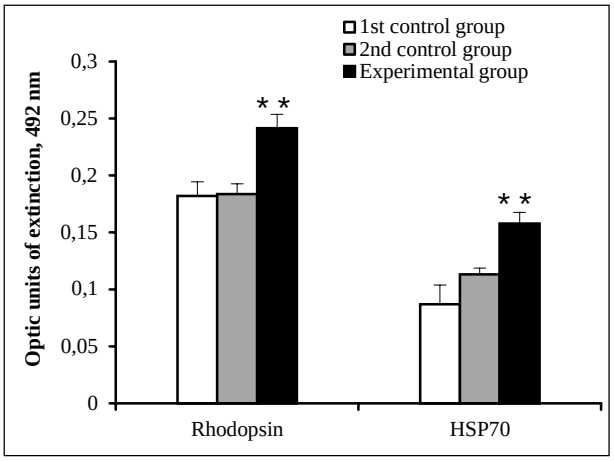

Figure 4. Effect of intra-vitreal administration of anti-SMAP antibodies after i.v. administration of MIAA on the levels of rhodopsin and HSP70 in the retinas of the rabbits (n=4, 8 eyes). ** - $p<0.01$.

In the 5th series of studies the effect of the anti-SMAP autoantibodies on the level of HSP70 and rhodopsin in the retina of the rabbits was studied. As a result of immunization with SMAP, the anti-SMAP antibodies of high titer were produced in the rabbits' organisms and they realized blocking effect on the activity of SMAP in all tissues. In the retina of the immunized rabbits significant upregulation of HSP70 relatively to the intact animals was revealed. Notably, in the intact animals (n=3; 6 eyes) the level of HSP70 in the retina made 0.087±0.01 optic units, as a result of immunization its level was upregulated to 0.176±0.01 optic units ($p<0.001$; Fig. 6). In addition, in the retinas of the SMAP-immunized animals upregulation of rhodopsin was noticed: 0.186±0.005 and 0.213±0.004 optic units in the intact and immunized animals, correspondently ($p<0.01$).

In the 6th series of studies the levels of natural anti-SMAP autoantibodies in the blood serum of the patients with retinitis pigmentosa were evaluated. The blood serum was used as the first antibodies in the indirect ELISA-test. Natural autoantibodies to all antigens are revealed normally in the healthy organism of the animals and humans (Avrameas, 1991; Poletayev, 1995; Lacroix-Desmazes et al., 1998). Hence, on basis of the revealed titers of the natural autoantibodies to certain antigens, one can make a conclusion concerning the levels of these antigens in the tissues.

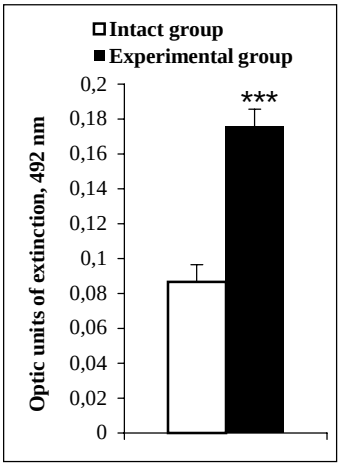

Figure 5. Effect of anti-SMAP autoantibodies (4-month immunization with SMAP) on the level of HSP70 in the retinas of the rabbits (n=3, 6 eyes). ** - $p<0.01$

In each of 9 patients, retinitis pigmentosa was diagnosed in clinical conditions by recording ERG. As a result, ERG was not recorded in any of 9 examined patients that indicates to a serious impairment of the receptor apparatus of the retina, i.e. retinitis pigmentosa.

The conducted studies revealed that in the blood serum of the patients with diagnosis of retinitis pigmentosa the level of natural anti-SMAP autoantibodies was significantly lower than in the healthy persons of the same age. Particularly, if the level of

natural anti-SMAP autoantibodies in the blood serum of the healthy persons (n=9) made 0.106±0.008 optic units, in the patients (n=9) their level made 0.076±0.004 optic units ($p<0.01$ on Student's t-criterion; Fig. 6).

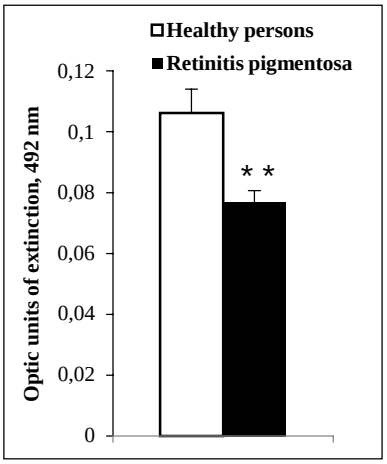

Figure 6. Changes of the level of natural anti-SMAP autoantibodies in the serum of the patients with diagnosed retinitis pigmentosa (n=9).
** - $p<0.01$.

On these grounds one can make a conclusion on downregulation of SMAP in the hypothalamus and retina of the patients with retinitis pigmentosa.

DISCUSSION

Finalizing the results of the conducted seven series of studies on the model of grave form of retinitis pigmentosa, the following conclusions might be made. Modeling grave form of retinitis pigmentosa on the rabbits through i.v. administration of MIAA at a high dose induces noticeable changes in a character of functioning of the receptor apparatus of the retina. In this case, sharp decline in the amplitudes of total ERG and downregulation of rhodopsin are noticed. In addition, in the formation of retinitis pigmentosa a number of molecular changes in the retina, apparently, underlying initiation and realization of regulatory processes both in the retina itself and outside it, in particular, in hypothalamus, are observed. Particularly, along with downergulation of rhodopsin, upregulation of HSP70 in the retina and upregulation of SMAP in the hypothalamus are revealed.

Sequence and cause and effect relations of these molecular events become apparent when we consider the results of other series of the studies as well. In particular, in the 3rd series of studies intra-vitreal administration of SMAP induces 23-fold upregulation of HSP70 in the retina that indicates to a capacity of SMAP in inducing powerful synthesis of this chaperon protein. Conversely, simultaneous upregulations of HSP70 and rhodopsin in the retina of the animals with grave form of retinitis pigmentosa under intra-vitreal administration of SMAP give grounds to making a conclusion that the nuclei of hypothalamus realize trophic regulation of the receptor apparatus of the retina, in particular, maintaining rhodopsin in a functionally active conformation through keeping baseline activity of serotonergic system in the hypothalamus, inducing in downstream way synthesis of HSP70 in the retina. Increase of the amplitude of total ERG under intra-ventricular administration of SMAP to the rabbits with retinitis pigmentosa supports the conclusion in regards to the trophic effects of SMAP toward the rhodopsin.

Antibodies-mediated blockade of SMAP activity, administered locally, into the corpus vitreous of the eyes of the animals with previously formed retinitis pigmentosa, induces noticeable upregulation of HSP70 and rhodopsin in the retina. These data indicate that anti-SMAP antibodies are captured by the retina cells from the corpus vitreous and, apparently, through anterograde axonal transport are delivered to the hypothalamic nuclei, wherein through antigen-antibody reaction they block the molecules of SMAP. In the literature there are data on the existence of direct retino-hypothalamic pathways capable of trophic regulation of receptor cells of the retina (Reuss, Fuchs, 2000; Trachtman, 2010). On the basis of the principle of negative biofeedback, compensatory synthesis of SMAP is initiated and its molecules through retrograde axonal transport are delivered from the hypothalamus to the retinal cells, wherein they launch mighty synthesis of HSP70. HSP70, due to their chaperon nature (Daugaard et al., 2007; Qu et al., 2015), recover normal conformation of the rhodopsin, disturbed by high dose of MIAA. Moreover, systemic blockade of SMAP with auto-antibodies, produced as a result of im-

munization of the rabbits, probably, through the mentioned above mechanism leads to significant upregulation of HSP70 and rhodopsin in the retina. Perhaps, this mechanism underlies getting the information about the functional status of receptor molecules of the retina by the engaged hypothalamic nuclei, and further adequate tuning on the system of its regulatory and synthetic activity in order to maintain receptor molecules in active conformational state occurs.

The revealed declined titer of natural anti-SMAP autoantibodies in the patients with retinitis pigmentosa, reflecting, correspondently, downregulation of SMAP in the organism's tissues, as well as the results of other series of the studies, conducted on the rabbits, provide grounds to put forward a conjecture that formation of this retinal pathology in the patients might be launched by insufficient synthesis of SMAP in the hypothalamic nuclei and poor realization of trophic support of the receptor apparatus of the retina.

This idea, in particular, is supported indirectly by the results of the 1st and 2nd series of studies, which revealed downregulation of amplitude of ERG and rhodopsin level in the retinas of the animals with retinitis pigmentosa and following spontaneous recovery of normal amplitude of ERG with time course.

In addition, possible existence of the described mechanism of retinitis pigmentosa as well indicates to compensatory upregulation of SMAP in the hypothalamus on the background of retinitis pigmentosa in the 2nd series of studies, as well as the results of the 1st and 4th series of the studies, wherein administration of SMAP, correspondently, into the brain lateral ventricle and corpus vitreous of the animals, significantly alleviated the manifestations of retinitis pigmentosa and promoted recovery of the disturbed functions of the retina (upregulation of declined amplitude of ERG and rhodopsin level).

Hence, the consideration of the results in a whole provides grounds to support the idea, proposed earlier by other authors, concerning mechanism of development of retinitis pigmentosa, related to impairment of trophic support of the retina, realized permanently by the hypothalamic nuclei.

ACKNOWLEDGEMENT

The author appreciates highly the creative and research role of her supervisor Arif Mekhtiev in planning and carrying out the studies, presented in the article, and the consultative support of Afat Nasibova in the studies on the human serum.

REFERENCES

Agayev T.M., Gadjiyeva N.A., Dmitrenko A.I., Rzayeva N.M. (2004) Neurophysiological studies of role of hypothalamus in mechanism of regulation of functions of different portions of visual analyzator. Baku. Elm.

Allahverdiyeva T.N., Mehdiyev Kh.Sh., A.A.Gaisina, A.A.Mekhiev. (2019) Serotonin-modulating aniconsolidation protein promotes survival of animals of different species under bacerial and chemical toxins. *J. Evol. Biochem. Physiol.,* **54 (1):** 7-10.

Avrameas S. (1991) Natural autoantibodies: from "horror autotoxins" to "gnothi seaton". *Immunology Today,* **12:** 154-158.

Catty D. (1989) Antibodies. **Volume II:** A practical approach. Oxford: Oxford University Press, 280 p., pp. 259.

Daiger S.P., Sullivan L.S., Bowne S.J. (2013) Genes and mutations causing retinitis pigmentosa. *Clin. Genet.,* **84(2):** 1-16. doi: 10.1111/cge.12203.

Daugaard M., Rohde M., Jaattela M. (2007) The heat shock protein 70 family: Highly homologous proteins. *FEBS Lett.,* **581:** 3702-3710.

Katsnelson A.B. (1958) Abnormalities of development and diseases of eyes in early childhood age. Moscow. Medicine.

Lacroix-Desmazes S., Kaveri S.V., Mouthon L., Ayouba A., Malachere E., Coutinho A., Kazaychkine M.D. (1998) Self-reactive natural autoantibodies in healthy individuals. *J. Immunology Methods,* **216:** 117-137.

Mekhtiev A.A. (2000) Revealing in the brain of albino rats the protein possessing anticonsolidation properties. *Bulletin Experim. Biol. Med.,* **129(8):** 147-150.

Ohguro H., Hooser J.P., Milam A.H., Palczewski K. (1995) Rhodopsin phosphorylation and dephosphorylation *in vivo. J. Biol. Chem.,* **270:** 14259–14262.

Osterman L.A. (1985) Chromatography of proteins and nucleic acids. Moscow: Nauka, pp. 536.

Poletayev A.B. (1995) Regulatory autoantibodies. In book: *Monoclonal Antibodies in Neurobiology*. Novosibirsk, pp. 37-47.

Qu B., Jia Y., Liu Y., Wang H., Ren G. (2015) The detection and role of heat shock protein 70 in various non-disease conditions and disease conditions: a literature review. *Cell Stress Chaperones*, **20:**885–892.

Reuss S., Fuchs E. (2000) Anterograde tracing of retinal afferents to the tree shrew hypo-thalamus and raphe. *Brain Research*, **874:** 66–74.

Trachtman J.N. (2010) Vision and hypothalamus. *Optometry*, **81:** 100-115.

Xiao T., Xu K., Zhang X., Xie Y., Li Y. (2019) Sector retinitis pigmentosa caused by mutations of the *RHO* gene. *Eye*, **33:** 592-599.

Eksperimental modeldə və klinikada torlu qişanın piqmentli distrofiyasının əsasında duran mexanizmlərin tədqiqi

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Bu məqalə ada dovşanlarında və torlu qişanın piqmentli distrofiyası diaqnozu müəyyən olunan xəstələrdə torlu qişanın piqmentli distrofiyasının molekulyar patogenetik mexanizmlərinin öyrənilməsinə həsr olunmuşdur.. Tədqiqatlar vivarium şəraitində saxlanilan 2,2-2,7 kq çəkisi Şinşilla cinsi olan erkək ada dovşanın üzərində aparılmışdır. Tədqiqatlarda torlu qişanın piqmentli distrofiyası olan xəstənin damarından götürülmüş qan nümunələri istifadə olunurdu. Torlu qişanın piqmentli distrofiyasini yaratmaq üçün dovşanların damarına monoyodosirkə turşusu (MYST, distrofiyanın ağır dərəcəsi, 1 kq heyvan kütləsinə 26 mq MYST) yeridilmişdir. Dolayı immuno-enzim analiz üsulu ilə hipotalamusda SMAZ-ın (Мехтиев, 2000), torlu qişada isə - rodopsin və 70 kDa molekulyar kütləsi olan istilik şoku zülallarının (İŞZ70) səviyyəsi, xəstələrin qan zərdabında - SMAZ-a qarşı təbii autoanticismlər müəyyən edilirdi. Nəticələr Studentin t-kriteriyasi ilə analiz olunmuşdur. MYST yeridilmiş dovşanlarda torlu qişada rodopsinin səviyyəsinin azalması ($p<0,001$), İŞZ70 səviyyəsinin artması ($p<0,001$) və hipotalamusda SMAZ səviyyəsinin artması ($p<0.01$) müşahidə olunmuşdur. Torlu qişanın piqmentli distrofiyası formalaşmış heyvanlarda SMAZ-in intravitreal daxil edilməsi torlu qişada rodopsinin ($p< 0,001$)və İŞZ70-in ($p<0.001$) səviyyələrinin nəzərə çarpan dərəcədə artmasına gətirir. Torlu qişanın piqmentli distrofiya diaqnozlu xəstələrin qan zərdabında SMAZ-a qarşı təbii autoanticisimlərin səviyyəsi sağlam test olunanlardan nəzərə çarpacaq dərəcədə azalmışdır ($p<0,001$). Məqalədə hipotalamusun torlu qişanın reseptor aparatına retroqrad və anteroqrad aksonal nəqliyyat hesabına mövcud olunmuş trofik təsirinin molekulyar mexanizmlərinin analiz olunmuşdur.

Acar sözlər: *Torlu qişanın piqment distrofiyası,dovşanlar, serotonin-modullu antikonsolidasiya olunmuş zülal (SMAZ), rodopsin, SMAZ-a qarşı anticismlər, torlu qişa, hipotalamus, SMAZ-a qarşı təbii autoanticismlər, 70 kDa molekulyar kütləsi olan istilik şoku zülalları*

Исследование подлежащих механизмов пигментной дистрофии сетчатки в экспериментальной модели и клинике

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Статья посвящена изучению подлежащих молекулярных механизмов пигментной дистрофии сетчатки у кроликов и у больных с данным заболеванием. Исследования выполняли на кроликах породы Шиншилла с массой тела 2.2-2.6 кг. Сыворотку получали от больных с пигментной дистрофией сетчатки. Пигментную дистрофию моделировали на кроликах посредством внутривенного введения монойодуксусной кислоты (МЙУК; тяжёлая степень, 26 мг/кг массы тела). Методом непрямого иммуноферментного анализа в гипоталамусе определяли уровень серотонин-модулируемого антиконсолидационного белка (СМАБ, Мехтиев, 2000) и белков теплового шока с мол. массой 70 кДа (БТШ70): в сетчатке – родопсина, а в сыворотке больных – естественных аутоантител к СМАБ. Результаты были проанализированы по t-критерию Стьюдента. Было обнаружено значительное снижение уровня родопсина ($p<0.001$) и повышение уровня БТШ70 ($p<0.001$) в сетчатке так же, как и повышение уровня СМАБ ($p<0.01$) в гипоталамусе у кроликов, которым предварительно вводили МЙУК. Интравитреальное введение СМАБ кроликам с предварительно введённой МЙУК приводило к значительному увеличению уровня родопсина ($p<0.001$) и БТШ70 ($p<0.001$) в сетчатке. В сыворотке больных с диагностированным пигментным ретинитом сетчатки было выявлено заметное снижение титров естественных аутоантител к СМАБ, относительно здоровых лиц того же возраста ($p<0.01$). В статье анализируются молекулярные механизмы, лежащие в основе гипоталамического трофического регуляторного влияния в отношении рецепторных клеток сетчатки посредством ретроградного и антероградного аксонного транспорта.

Ключевые слова: *Пигментная дистрофия сетчатки, кролики, серотонин-модулируемый антиконсолидационный белок (СМАБ), родопсин, белки теплового шока с мол. массой 70 кДа, антитела к СМАБ, сетчатка, гипоталамус, естественные аутоантитела к СМАБ*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 140-144 (2020)

Effects of fluoxetine on memory processes in the rats with different phenotypes of nervous system and different levels of biogenic monoamines of the brain

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Accepted for publication: 17 April 2020

The present paper explores the effects of the psychopharmacological agent - fluoxetine - on mnestic processes, using a model of passive avoidance on male Wistar rats with different nervous system phenotypes and different activity ratios of the monoaminergic systems of the brains. In the re-test session under administration of fluoxetine, the seizure-tolerant rats compared to the seizure-sensitive rats were characterized by a more pronounced fear response to the "unsafe" compartment and enhanced anxiety facilitating the retention of memory trace. The individual sensitivity of the animals to the action of fluoxetine and the direction of its effects on mnestic processes are supposed to be determined by different primary activity ratios of the monoaminergic systems of the brain.

Keywords: *Seizure-tolerant and seizure-sensitive rats, passive avoidance response, fluoxetine, serotonin, dopamine, noradrenaline*

INTRODUCTION

In recent years, increasing attention has been paid to the study of the functional specificity of the central nervous system (CNS), determined by both genetic (different strains of rats) and individual (differences within one strain) peculiarities of behavior, memory, learning, adaptation and plasticity. It is known, that the individual reactivity of organism to the action of different stress-factors are associated with the innate difference in activities of the monoaminergic (MA) systems of the brain, involved in the neurochemical organization of various types of innate and learned behaviors (Ismayilova et al., 2007). In this regard, the most significant neurotransmitter is serotonin (5-HT) being an important biochemical factor forming mixed anxiety-depressive disorders and disturbing cognitive functions (Handley and Mcblune, 1993). In particular, deficiency of 5-HT leads to a disturbance of synaptic transmission in the CNS and forms depressive states. Therefore, most psychotropic medications applied in medical practice are targeted at enhancing serotonin neurotransmission. Among the medications that affect intra-synaptic serotonin metabolism, the selective serotonin reuptake inhibitors, such as fluoxetine, play a key role (Wong et al., 2005). The medication binds to the specific protein – serotonin transporter – selectively blocking serotonin reuptake in the presynaptic ending, which leads to increase in concentration of the neurotransmitter in the synaptic cleft and to enhancing its action on the postsynaptic receptors.

A lot of scientific papers are dedicated to the comparative study of the effects of acute and chronic administration of fluoxetine on behavior in various models of rats and mice of different strains (Kovalenko et al., 2007; Sarkisova and Folomkina, 2010). In addition, they contain the information about the variety of the neuro-psychotropic medications, that depends on the animal genotype, nature of the test conditions (Griebel et al., 2000) and the baseline psycho-emotional state of the individuals (Ben Porath and Taylor, 2002).

Based on the aforementioned, of particular interest is to study the effects of 5-HT excess caused by fluoxetine on the mnestic processes, using a model of passive avoidance (PA) on male Wistar rats with different nervous system phenotypes and different activity ratios of the catecholaminergic and

doi.org/10.29228/jlsb.52

5-HT-ergic systems of the brain. Passive avoidance test is one of the main techniques of testing neuropsychotropic medications' effects and, moreover, it is especially popular in studying mnestic process patterns (Voronina and Seredenin, 1998).

MATERIALS AND METHODS

The study was carried out on male Wistar rats (body mass of 180-220 g) under chronic conditions. The rats were preliminarily tested for tolerance to acoustic startle stimulus. For that purpose, each animal was exposed to the sound of an electric bell (90-120 dB) for 2 min in the soundproof box. The indicator of sensitivity was the intensity of seizure in the rats. The difference in the responses to acoustic stimulus allowed dividing the animals into 2 groups: seizure-sensitive (SS – prone to seizures) and seizure-tolerant (ST – without motor excitation) rats.

From the total number (121) of the rats, 29 ST and 27 SS rats were selected. Both types of the animals were divided into the experimental and control animals. 1 h prior to the experiment, the experimental animals (ST (n=15), SS (n=14)) were intraperitoneally injected with fluoxetine (Pharmascience, Montreal, Canada) at a dose of 25mg/kg. The control rats (ST (n=14), SS (n=13)) were administered with the diluent - distilled water - in the equal volume. During 2 days prior to the main experiments, the animals were handled for 5 min per day in order to equalize their responses to this stimulus.

PA-elaboration was carried out according to the common technique in the light-dark box. The rats were placed in the light compartment with their tails to the guillotine door between the light and dark compartments. The latency to enter the dark compartment was recorded (unconditioned "mink" reflex). When the animal entered the dark compartment, the guillotine door was closed and a mild electric foot shock (0.5 mA; 2 sec) was delivered through the grid floor. Then the animals were quickly removed. The stability of the formed response was characterized by the degree of its retention in the re-test session on the 2nd day, which allowed identifying the peculiarities of the memory traces retention. The time spent by the animals in the light "safe" compartment was recorded for 300 sec. The behavioral (search movements, rearing, grooming) and vegetative (number of fecal boluses) indices registered in PA re-test session were also analyzed.

While processing the experimental material, we have considered the total time spent by the rats in the light compartment and the number of rats that retained the formed PA response, as well as analyzed the range of behavioral (search movements, rearing, grooming) and vegetative (number of fecal boluses) indices in PA re-test session.

All the experimental procedures were carried out in accordance with the international and national standards for the care and use of laboratory animals and approved by the appropriate committee of the Institute of Physiology, ANAS. The results of the study were processed with application of a nonparametric Mann–Whitney U test and Student's t-test. Mathematical calculations were performed using an analytics software package – STATISTICA.

RESULTS AND DISCUSSION

The comparative analysis of learning in the animals with different proneness to seizures identified the peculiarities of PA response retention in the re-test session on the 2nd day after training. It has been found that the control ST rats compared to the SS ones had lower rate of PA response retention (17.8 and 22.4 % respectively, $p<0.05$). However, under administration of fluoxetine, the lower rate of response retention was observed in the SS rats compared to the ST ones (12.9 % and 53.2% respectively, $p<0.01$) (Table 1). The number of entries to the dark compartment was larger in the SS rats compared to the ST ones. Thus, one part of the SS rats entered and left the dark compartment for several times, while the other part entered immediately the dark compartment and stayed there until the end of the experiment, demonstrating an impairment of retention of the formed response.

The total time spent in the "safe" compartment on the 2nd day after training in the control ST rats made up 189.2±0.6 sec on average, which was significantly lower ($p<0.01$) than the total time spent by the SS rats in the light compartment – 283.6±0.9 sec (Table 2).

Table 1. Retention of PA response (%) under administration of fluoxetine in the rats with different levels of proneness to seizures

Groups	Control	Experimental
ST rats	17.8	53.2*
SS rats	22.4	12.9**

*p < 0.05; **p < 0.01.

Table 2. The total time (sec) spent in the "safe" compartment on the 2nd day after PA response elaboration under administration of fluoxetine in the rats with different levels of proneness to seizures

Groups	Control	Experimental
ST rats	189.2	230.5
SS rats	283.6	122.2 **

**p < 0.01

However, acute administration of fluoxetine led to the opposite effects on memory traces retrieval in the experimental animals of both types. Under administration of the medication, high rate of retention of the formed PA response in the re-test session on the 2nd day was identified in the ST rats compared to the control ones. That was manifested in increase in the total time spent in the light compartment – 230.5±0.7 sec, while in the SS rats, there was significant decrease in the mentioned parameter – 122.2±0.6 sec (p< 01).

Under administration of the medication, the differences in PA response retention capacity of the rats of both types were more pronounced in the context of the number of animals that retained the formed response. Thus, on the 2nd day after training, the share of the control SS rats that retained the response made up 83%, while in the ST rats it was 43%. However, under administration of fluoxetine, that parameter made up 40% in the SS rats and 60% in the ST ones.

The analysis of the range of behavioral and vegetative indices accompanying the PA response in the re-test session on the 2nd day showed the behavioral differences between 2 experimental groups of the animals administered with fluoxetine (Fig. 1). There were enhanced search activity and low level of the vegetative indices in the control SS rats, whose time spent in the light compartment was longer in comparison to the ST rats. Under administration of the medication, high rate of PA response retention was observed in the ST rats, manifested in increase in the total time spent by the animals in the "safe" compartment, enhanced search activity and low level of the vegetative index. In the SS rats compared to the control ones, those parameters were lower. However, under the effects of fluoxetine there was a completely opposite pattern of memory traces retrieval in the experimental group of both animal types. Under administration of the medication, high rate of PA response retention on all days of testing were identified in the ST rats. That was manifested in increase in the total time spent by the animals in the "safe" compartment and the level of search activity. In the SS rats compared to the control, there was decrease in the mentioned parameters.

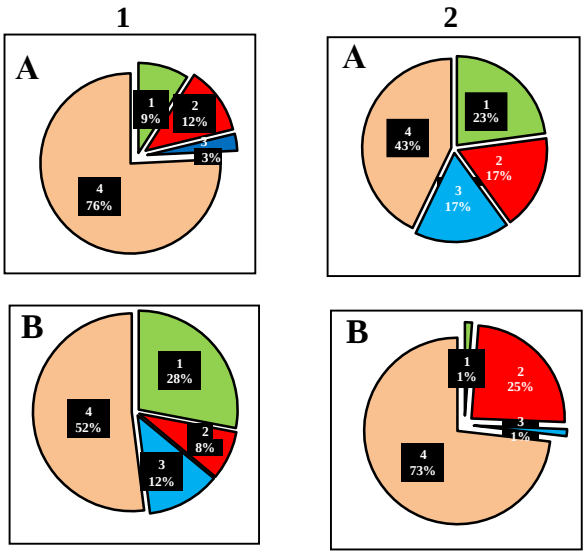

Fig. 1. Range of the behavioral and vegetative indices revealed in PA re-test session on the 2nd day after training in the rats with tolerance (1) and sensitivity (2) to acoustic startle under administration of fluoxetine. A – control group; B – experimental group. Numbers on the sectors of the circles indicate the manifestation degree (%) of some behavior's components: 1 – search activity; 2 - grooming; 3 - rearing; 4 – fecal boluses.

Thus, under the effects of administration of the medication, the ST rats compared to the SS rats are characterized by a more pronounced fear response to the "unsafe" compartment and enhanced anxiety facilitating the formation of long-term memory traces and showing individual sensitivity of the animals to the action of fluoxetine on mnestic processes. The differences in the processes of memory traces retrieval under the effects of fluoxetine in the animals of

different phenotypes are apparently supposed to be due to the impact of the medication on metabolism of monoamines, which changes an innate activity ratio of the noradrenaline (NA)-, dopamine (DA)- and serotonin (5-HT)-ergic systems of the brain. The manifestation degree of the effects of the medication depends on both the individual specificity of the CNS and the specific brain area. Thus, acute administration of fluoxetine identified the response peculiarities of the MA-ergic systems of various brain areas to its effects (Ismayilova et al., 2016). In particular, after administration of the medication, there was significant decrease in 5-HT level in the hypothalami of the SS rats, as well as significant increase in NA level, which led to PA response extinction. The aforementioned is substantiated by the data that Wistar rats with different phenotypic peculiarities of the nervous system, whose activity ratio of the MA-ergic systems shifted toward the predominance of the 5-HT-ergic system of the brain, have the best ability to retain PA response (Semenova, 1992). However, under the effects of fluoxetine, in the ST rats, there was significant increase in 5-HT level in the frontal cortex accompanied by decrease in NA level and significant decrease in DA level, which led to PA response recovery. The obtained data is consistent with the opinion of R.I. Kruglikov (5) on increasing time spent in the "safe" compartment during reducing NA in the brain by disulfiram. In addition, our data is corroborated by the works of many investigators (Hervas et al., 2001), indicating increase in 5-HT level in the frontal cortex after administration of fluoxetine at a dose of 3-154 mg/kg, as well as an inhibitory effect of increased 5-HT level on the DA-ergic system (Fletcher et al.,1999).

Thus, extinction of mnestic processes, observed in our studies, under the effects of the medication in the SS rats is probably associated with weakening genetically determined activity of the 5-HT-ergic system of the hypothalami while a better retention of memory traces in the ST rats is correlated with increased 5-HT- ergic and decreased NA-ergic systems' activity of the frontal cortex.

The individual sensitivity of the animals to the action of the psychopharmacological agent – fluoxetine – and the direction of its effects on mnestic processes are supposed to be determined by different primary activity ratios of the MA-ergic systems of the brain.

REFERENCES

Ben-Porath D.D., Taylor S.P. (2002) The effects of diazepam (valium) and aggressive disposition on human aggression: an experimental investigation. *Addict. Behav*, **27(2):** 167-177; DOI: 10.1016/s0306-4603(00)00175-1

Fletcher P.J., Korth K.M., Chambers I.W. (1999) Selective destruction of brain serotonin neurons by 5,7- dihydroxytryptamine increases responding for a conditioned reward. *Psychopharmacology*, **147:** 291-299; DOI: 10.1007/s002130051170

Griebel G., Belzung C., Perrault G., Sanger D.J. (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology*, **148:** 164-170; DOI: 10.1007/s002130050038

Handley S.L., McBlune J.W. (1993) Serotonin mechanisms in animal models of Anxiety. *Brasil J. Med. Biol. Res.*, **26(1):** 1-13.

Hervas I., Vilaro M.T., Romero L., Scorza MC., Mengod G., Artigas F. (2001) Desensitization of 5-HT_{1A} autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. *Neuropsycho-pharmacology*, **24:** 11-20; DOI: 10.1016/S0893-133X(00)00175-5

Ismayilova Kh.Yu., Aghayev T.M., Semenova T.P. (2007) Monograph: Individual peculiarities of behavior (monoaminergic mechanisms). (monograph) *Baku: Nurlan Press*, 228 p. (in Russian).

Ismayilova Kh.Yu., Majidi M.B., Jalilova A.L. (2016) The effects of fluoxetine on aminergic indices of anxiety in rats with different nervous system phenotypes. *Proceedings of the Azerbaijan NAS (biol. and med. sci.)*, **71(2):** 69-75 (in Azerbaijani).

Kovalenko I.L., Avgustinovich D.F., Tolstikova T.G. (2007) The effects of acute and chronic administration of fluoxetine in male and female mice with mixed anxiety-depressive disorders. *Russian Journal of Physiology*, **12:** 1401-1412 (in Russian).

Kruglikov R.I. (1989) Neurochemical basis of learning and memory. *Moscow: Nauka*, 160 p. (in Russian).

Sarkisova K.Yu., Folomkina A.A. (2010) The effects of the selective serotonin reuptake inhibitor

– fluoxetine – on symptoms of depressive-like behavior in rats of WAG/Rij strain. *Journal of Higher Nervous Activity*, **60(1):** 98-108 (in Russian).

Semenova T.P. (1992) Optimization of learning and memory processes. (monograph) *Pushino:* 152 p. (in Russian).

Voronina T.A., Seredenin S.B. (1998) Nootropic medications, achievements and new challenges. *Experimental and clinical pharmachology*, **56(4):** 3-9 (in Russian).

Wong D.T., Perry K.W., Bymaster F.P. (2005) Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat. Rev. Drug Discov.*, **4(9):** 764-774; DOI: 10.1038/nrd1821

Müxtəlif fenotipli sinir sistemi və beynin bıogen monoaminlərinin səviyyəsinin müxtəlifliyi ilə fərqlənən siçovullarda fluoksetinin yaddaş proseslərində effektləri

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Müxtəlif fenotipli sinir sistemi və beyində monoaminergik (MA) sistemlərinin nisbətinin müxtəlifliyi ilə fərqlənən siçovullarda passiv xilasolma şərti refleksi modelindən istifadə etməklə, fluoksetinin mnestik proseslərə təsiri öyrənilmişdir. Psixofizioloji preparatın yeridilməsi fonunda şərti reflektor reaksiyanın test edilməsinin ikinci günündə alınan nəticə stressə-tolerant siçovulların stressə-həssas siçovullardan fərqli olaraq, "təhlükəli" bölmə qarşısında kəskin qorxu hissi keçirilməsi və yüksək həyəcanlı olması yaddaş izinin saxlanıldığının təsdiqi ola bilər. Psixofarmakoloji preparat olan fluoksetinin təsirinə qarşı heyvanın fərdi həssaslığı və mnestik proseslərdə onların yaratdığı effektin istiqamətinin beynin müxtəlif strukturlarında MA-ergik sistemlərin müxtəlif ilkin nisbəti ilə əlaqədar olduğu güman edilir.

Açar sözlər: *Stresə qarşı qıcolma-tolerant və qıcolma-həssas siçovullar, passiv xilasolma şərti refleksi, fluoksetin, serotonin, dofamin, noradrenalin*

Эффекты флуоксетина в процессах памяти у крыс с различным фенотипом нервной системы и разным уровнем биогенных моноаминов мозга

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С использованием модели условного рефлекса пассивного избегания, у крыс-самцов линии Вистар с различным фенотипом нервной системы и различным соотношением активности моноаминергических (МА) систем мозга изучалось влияние флуоксетина на процессы памяти. В отличие от судорожно-чувствительных крыс, судорожно-толерантные к стрессу крысы на фоне введения психофармакологического препарата характеризовались более выраженной реакцией страха перед "опасным" отсеком и повышенной тревожностью на второй день тестирования условнорефлекторной реакции, что может указывать на сохранение следа памяти. Предполагается, что индивидуальная чувствительность животного к действию психофармакологического препарата флуоксетина и направленность вызываемых им эффектов в мнестических процессах обусловлены различным исходным соотношением активности МА-систем различных структур мозга.

Ключевые слова: *Судорожно-толерантные и судорожно-чувствительные к стрессу крысы, условный рефлекс пассивного избегания, флуоксетин, серотонин, дофамин, норадреналин*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 145-150 (2020)

Study of the effect of compositions of mineral oils with ether oils on the mosquitoes

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Accepted for publication: 05 June 2020

The purpose of our study was elaboration of locally applied phytopreparations capable of providing effective protection from mosquitoes. Unique oils of Azerbaijan are rich in naphthenic hydrocarbons, so they are suitable for preparing cosmetic and medical oils. We have obtained cosmetic oils by fully purifying oil distillates from aromatic hydrocarbons in presence of natural and synthetic adsorbents. The composition of purified oil distillates of essential oil, obtained from the plants and their mixtures, has been studied. Though rubbing of the hands with the control oil distillate did not have any result, application of the prepared compositions of oils gave positive results in protection from mosquitoes.

Keywords: *Oil distillates of petroleum, essential oils, protection from mosquitoes*

INTRODUCTION

There are over 3,000 species of mosquitoes in the world, 500 species of them are malaria mosquitoes. 70 species of these mosquitoes spread malaria and other parasitic diseases among people (Lysenko, 2003).

28 kinds of mosquitoes of 7 species dwell in the regions of our Republic, 7 of them are malaria and 21 are non-malaria mosquitoes (Baghirov and Aliyev, 2012). These malaria and non-malaria mosquitoes can spread various parasitic and infectious diseases, viruses and arboviruses among people.

As a result of the researches, the antibodies to Tyaginya arbovirus from Aedes vexans mosquitoes in Sabirabad region (Gaidamowitz, 1968) and from Anopheles hyrcanus mosquitoes in Lankaran region were found in the blood of local population (Lysenko, 2003).

In Qyzylaghaj reserve, the Uukuniemi arbovirus was obtaines from Culex mosquitoes and in addition, antibodies to Sindbis and Tyaginya arboviruses were found in the blood of the local population (Ismailov, 2008).

Also, in 2013, the Sindbis arbovirus was revealed in this zone from Anapheles Maculipennis mosquitoes, caught in a non-residential building in Garagurd village of Khachmaz region (Aliyev, 2013).

An.vexans, C.modestus, An.hyrcanus və An.maculipennis mosquitoes can spread parasitic disease - Tularemia in our Republic. Ahopheles, Culex, Aedes and other mosquito species spread Filaria parasites among people (Gaidamowitz S.Y., 1971).

So, because of the need of preparing a local, natural and completely harmless preparation against mosquito in our Republic, we have elaborated a medicine that has an effect in protecting from mosquitoes and that has been tested in laboratory conditions Gurinovich и Puchkova, 2005; Kyazimov, 2005).

MATERIAL AND METHODS

For the purpose of studying the effect of various oils against mosquitoes, the researchers of the Institute of Petrochemical Processes named after

doi.org/10.29228/jlsb.53

Academician Y.Mammadaliyev prepared the drug preparation, based on combination of White Naphthalan oil with 5 oils (thuja, eucalyptus, rosemary, mint, pine) at certain ratios. The strong bactericidal effect of the preparation was also studied on 4 microbes: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* bacteria and *Candida alpicans* fungus.

Reagents used in the preparation of compositions:

The quality indicators of the White Naphthalan oil (fraction 260-500^{o}C fraction) corresponds to the table 1 on "White Naphthalan Oil TSh AZ 3536601.246-2016".

The quality indices of essential oils of rosemary, eucalyptus and ordinary pine plants are given in Table 2.

Physical and chemical parameters of preparations used in the preparation of compositions are given in Table 3 "White Naphthalan oil based cosmetic ointments TSh AZ 3536601.264-2018".

Table 1. Quality indices of White Naphthalan oil.

The name of index	Norm	Test methods
External appearance	Transparent colourless homogeneous fluid	According to the 4.2. point of current TSh
Density at 20ºC, kg/m^3, should not be less	860	According to ГОСТ 3900
Kinematic viscosity at 20ºC, mm^2/sec, should not be less	10	According to ГОСТ 31391
Ignition temperature ºC, should not be lower	90	According to ГОСТ 4333
The amount of aromatic hydrocarbons	There is no	According to ГОСТ 6994
The amount of resins	There is no	According to the 4.3. point of current TSh
Mass fraction of Naphthene hydrocarbons, with percent, should not be less	97	According to the 4.4. point of current TSh
Acid number to 1 g product, mg KOH	Absent	According to ГОСТ 5985
Amount of water	Absent	According to ГОСТ 2477
The amount of acids and solvents, soluble in water	Absent	According to ГОСТ 6307

Table 2. The quality indices of essential oils of rosemary, eucalyptus and ordinary pine plants

The name of indicators	Rosemary oil ГОСТ-31791-2017	Eucalyptus oil ОКП-91-5120	Ordinary Pine oil ОСТ-10-81-87
Viscosity, g/sm	0,875-0.905	0.870-0.920	0.868-0.903
Acid number, mg KOH/g, should not be too much	Should not exceed 1	Should not exceed 2	Should not exceed 2
Ester number, mg KOH	6.0-20.0	-	Should not be less than 15.0
Breakdown factor, 20^{0}C	1.4600-1.4750	1.459-1.470	1.458 -1.485

Table 3. Physical and chemical indices of preparations

The name of indicators	Norms according to marks			Test methods
	White Naphthalan+ Rosemary oil	White Naphthalan+ Eucalyptus oil	White Naphthalan + Ordinary Pine oil	
Iodine number, g Y/100 g, should not be more	0.722	1.225	1.177	According to ГОСТ 2070
Acid number, mg KOH/g, should not be more	Absent	0.02	Absent	According to ГОСТ 11362
Density, g/cm^3 20ºC, should not be less	0.8619	0.8628	0.8690	According to ГОСТ 3900
Kinematic viscosity , mm^2/sec, should not be less 40ºC 100ºC	 8.0505 1.5434	 5.0482 1.5849	 5.4306 1.7399	According to ГОСТ 31391
Ignition temperature ºC, should not be lower	75	75	95	According to ГОСТ 4333
Freezing temperature ºC, should not be above	-60°C			According to ГОСТ 20287
External appearance	Transparent colourless homogeneous fluid			According to ГОСТ 29188.0 (section 3)

To test the protective effect of the preparations from mosquitoes, researches have been carried out at the Scientific Research Institute of Medical Prevention named after Academician V.Akhundov. The studies were carried out in the way as recommended by the World Health Organization (WHO) and the authors have been added to this method (8).

In order to study the effects of oils on the *Cx.p.molestus* mosquitoes, the larvae and pups of mosquitoes were collected and brought into the laboratory in the water collected in the basement of the building 23 located in the Third Micro district. The delivered larvae and pups were collected and placed in the narrow cages of the desks, and the winged mosquitoes were taken for studies.

While carrying out the experiments, 30-40 *Cx.p.molestus* mosquito females were released into each of the 2 small desktop narrow cages of 30 X 30 X 30 cm. One of the cages has been selected for an experimental study and the other one – for control. The experiments have been repeated 3 times. During the experiment the temperature of the laboratory was recorded.

Oily hand (with essential oil) was included to the one of the cages, whereas oilless hand – to the second cage. The effects of essential oils on the mosquitoes were evaluated by recording the timeframe till they sucked blood. Exposure of the hand to mosquito's contact lasted about 2 h (for experimental hand) and 15 min (in control). The window of the laboratory was covered with black stuff. The results of the experiments are presented in the tables.

RESULTS AND DISCUSSION

As can be seen from the data, the ingredients which contain eucalyptus, rosemary and *Pinus sylvestris* L. oils have stronger repellent effects on mosquitoes.

As can be seen from Table 4, when is used a mixture containing 4% of Thuya oil and 96% of White Naphthalene oil mosquitoes do not approach to humans for up to 4 hours.

As can be seen from Table 5, when is used a mixture 4% of eucalyptus oil and 96% of White Naphthalene oil mosquitoes do not come close to humans for 5 hours.

When is used a mixture containing of 4% rosemary oil and 96% of white naphthalene oil mosquitoes do not come close to humans for 6 hours (Table 6).

When is used a mixture of 4% peppermint oil and 96% of white naphthalene oil mosquitoes do not come close to humans for 2 hours (Table 7).

As can be seen from Table 8, when is used 4% of *Pinus sylvestris* L. Oil and 96% of White Naphthalene Oil mosquitoes do not approach to humans for 6 hours.

On the other hand, our country has large natural resources to obtain *Pinus sylvestris* L., eucalyptus and rosemary oils.

Table 4. The effect of the composition (1) with Tuja (*Thuja orientalis* L.) oil on the *Culex pipiens molestus* mosquitoes.

Mosquitoes's		Temperature of the laboratory, ºC	Control to the experiments
Contact time	**Sucking blood during contact**		**Sucked blood during contact**
25.08.2017			
8^{00}-8^{15}	Did not suck blood	27.0	Sucked blood after 5 min
10^{00}-10^{15}	Did not suck blood	27.0	Sucked blood after 7 min
12^{00}-12^{15}	Sucked blood	27.4	Sucked blood after 4 min
28.08.2017			
8^{00}-8^{15}	Did not suck blood	27.4	Sucked blood after 4 min
10^{00}-10^{15}	Did not suck blood	27.6	Sucked blood after 3 min
12^{00}-12^{15}	Sucked blood	27.6	Sucked blood after 6 min
29.08.2017			
9^{00}-9^{15}	Did not suck blood	20.0	Sucked blood after 6 min
11^{00}-11^{15}	Did not suck blood	20.0	Sucked blood after 4 min
13^{00}-13^{15}	Sucked blood	20.2	Sucked blood after 5 min

Table 5. The effect of the composition (2) with Eucalyptus oil on the *Culex pipiens molestus* mosquitoes.

Mosquitoes's		Temperature of the laboratory, ºC	Control to the experiments
Contact time	**Sucking blood during contact**		**Sucked blood during contact**
30.08.2017			
8^{00}-8^{15}	Did not suck blood	27.4	Sucked blood after 6 min
10^{00}-10^{15}	Did not suck blood	27.4	Sucked blood after 4 min
12^{00}-12^{15}	Did not suck blood	27.6	Sucked blood after 4 min
13^{00}-13^{15}	Sucked blood after 4 min.	27.8	Sucked blood after 3 min
31.08.2017			
8^{00}-8^{15}	Did not suck blood	27.0	Sucked blood after 6 min
10^{00}-10^{15}	Did not suck blood	27.2	Sucked blood after 5 min
12^{00}-12^{15}	Did not suck blood	27.4	Sucked blood after 4 min
13^{00}-13^{15}	Sucked blood after 4 min.	27.6	Sucked blood after 4 min
2.10.2017			
8^{00}-8^{15}	Did not suck blood	19.8	Sucked blood after 5 min
10^{00}-10^{15}	Did not suck blood	19.8	Sucked blood after 6 min
12^{00}-12^{15}	Did not suck blood	19.8	Sucked blood after 4 min
13^{00}-13^{15}	Sucked blood after 10 min.	19.8	Sucked blood after 4 min

Table 6. The effect of the composition with Rosemary oil (*Ros arinus L.*) on the *Culex pipiens molestus* mosquitoes

Mosquitoes's		Temperature of the laboratory, ºC	Control to the experiments
Contact time	**Sucking blood during contact**		**Sucked blood during contact**
5.09.2017			
8^{30}-8^{45}	Did not suck blood	24.0	Sucked blood after 4 min
10^{30}-10^{45}	Did not suck blood	24.2	Sucked blood after 6 min
12^{30}-12^{45}	Did not suck blood	24.4	Sucked blood after 3 min
14^{30}-14^{45}	Sucked blood after 3 min.	24.4	Sucked blood after 2 min
7.09.2017			
8^{30}-8^{45}	Did not suck blood	24.0	Sucked blood after 6 min
10^{00}-10^{15}	Did not suck blood	24.0	Sucked blood after 4 min
12^{30}-12^{45}	Did not suck blood	24.2	Sucked blood after 4 min
14^{30}-14^{45}	Sucked blood after 4 min	24.2	Sucked blood after 3 min
3.10.2017			
9^{00}-9^{15}	Did not suck blood	18.0	Sucked blood after 5 min
11^{00}-11^{15}	Did not suck blood	18.0	Sucked blood after 3 min
13^{00}-13^{15}	Did not suck blood	18.0	Sucked blood after 4 min
15^{00}-15^{15}	Sucked blood after 3 min.	18.0	Sucked blood after 4 min

Table 7. The effect of the composition with Mint oil (*Mentha L.*) on the *Culex pipiens molestus* mosquitoes.

Mosquitoes's		Temperature of the laboratory, ºC	Control to the experiments
Contact time	**Sucking blood during contact**		**Sucked blood during contact**
12.09.2017			
8^{00}-8^{15}	Did not suck blood	26.2	Sucked blood after 5 min
10^{00}-10^{15}	Sucked blood	26.2	Sucked blood after 3 min
13.09.2017			
8^{00}-8^{15}	Did not suck blood	26.4	Sucked blood after 4 min
10^{00}-10^{15}	Sucked blood	26.4	Sucked blood after 5 min
21.09.2017			
8^{00}-8^{15}	Did not suck blood	26.4	Sucked blood after 4 min
10^{00}-10^{15}	Sucked blood	26.4	Sucked blood after 3 min

Table 8. The effect of the composition with Pine oil (*Pinus sylvestris* L.) on the *Culex pipiens molestus* mosquitoes.

Mosquitoes's		Temperature of the laboratory, ºC	Control to the experiments
Contact time	**Sucking blood during contact**		**Sucked blood during contact**
28.09.2017			
8^{00}-8^{15}	Did not suck blood	24.0	Sucked blood after 6 min
10^{00}-10^{15}	Did not suck blood	24.0	Sucked blood after 6 min
12^{00}-12^{15}	Did not suck blood	24.2	Sucked blood after 5 min
14^{00}-14^{15}	Sucked blood	24.2	Sucked blood after 4 min
29.10.2017			
8^{00}-8^{15}	Did not suck blood	23.0	Sucked blood after 5 min
10^{00}-10^{15}	Did not suck blood	23.0	Sucked blood after 6 min
12^{00}-12^{15}	Did not suck blood	23.2	Sucked blood after 4 min
14^{00}-14^{15}	Sucked blood	23.2	Sucked blood after 5 min
5.10.2017			
8^{30}-8^{45}	Did not suck blood	16.0	Sucked blood after 5 min
10^{30}-10^{45}	Did not suck blood	16.0	Sucked blood after 5 min
12^{30}-12^{45}	Did not suck blood	16.0	Sucked blood after 6 min
14^{30}-14^{45}	Sucked blood	16.2	Sucked blood after 6 min

CONCLUSION

As it is shown in the tables, the protective effect of the mixture of White Naphthalan oil with thuja and mint oil against mosquitoes was 2 h during the study. The protective effect of the mixture of eucalyptus oil, rosemary oil and ordinary Pine tree oil against mosquitoes made 5-6 h. Taking into account these data, it is important to study the effect of the mixture of White Naphthalan oil with eucalyptus oil, rosemary oil and ordinary Pine tree oil against mosquitoes in natural conditions.

REFERENCES

Bagirov H.A., Aliyev M.I. (2012) Fauna of blood-sucking mosquitoes in Azerbaijan (1771-1998). *Modern achievements of Azerbaijani medicine, №1: 94-96 (in Azerbaijan)*

Aliyev M.I., Abdullayev R., Ismayilov A. (2013) Types of mosquitoes caught in the Guba-Khachmaz region of the Greater Caucasus. *Scientific works of the National Institute of Medical Prophylaxis named after V.Akhundov*, VI: 22-26. (in Azerbaijan).

Gaidamowitz S.Ya., Klisenko GA,. Chervonsky V.I. (1971) Neutralizing antibodies to the virus Tahinia in the population of some districts of the Azerbaijan SSR. *Questions of medicine. virus. arbovirus., XI: 118-119 (in Russian)*

Gaidamowitz S.Y., Klisenko G.A., Chervonsky V.I (1971) Neutralizing antibodies to the virus Tahinia in the population of some districts of the Azerbaijan SSR. Questions of medicine. virus. arbovirus., **XI:** 118-119 (in Russian).

Gaidamowitz S.Y., Nikiforov L.P., Gromashevsky V.A (1968) Separation of arboviruses from groups A and B in Azerbaijan. *Proceedings of the XV scientific session of the Institute of Poliomyelitis and Viral Encephalitis AMN SSR*, **3:** 185-186. (in Russian)

Gurinovich L.K., Puchkova T.V. (2005) Essential oils: chemistry, technology, analysis and application. M.: School of Cosmetic Chemists, 192 p. (in Russian).

Ismailov A.Sh. (2008) Arboviruses and arbovirus infections - pathology and ecology. *Reviews and problematic articles, Biomedicine,* **4:** 3-9 (in Russian)

Kyazimov G.A. (2005) Characteristics of Naftalan oil and naftalan ointment. *In the world of medicine (Baku),* **1:** 71-74 (in Russian).

Lysenko A.Y., Kondrashin A.V., Yezhov M.N. (2003) Malaria, WHO. Regional Office for Europe, Copenhagen, p. 237-256 (in Russian)

Samedova F.I. (1996) Technology for producing white oils from Azerbaijani oil. Baku: Elm, 50-55 p. (in Russian).

Mineral yağlarin efir yağlari ilə kompozisiyalarinin ağcaqanadlara hürküdücü təsirinin öyrənilməsi

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Tədqiqatımızın məqsədi ağcaqanadlara hürküdücü təsir göstərən yerli və effektiv fitopreparatların işlənib hazırlanması olmuşdur. Azərbaycanın unikal neftləri əsasən naften karbohidrogenləri ilə zəngin olduğundan onlar kosmetik və tibbi yağlar alınması üçün əlverişlidir. Tərəfimizdən təbii və sintetik adsorbentlər iştirakı ilə yağ distillatlarını aromatik karbohidrogenlərdən tam təmizlənərək kosmetik yağlar alınmış və onların tərkibi öyrınilmişdir. Nəzarət variantda təmizlənmiş yağ distillatlarının ələ sürtülməsi nəticə verməsə də bitkilərdən alınmış efir yağlarının və onların qarışıqları ilə müvafiq müsbət nəticələr əldə olunmuşdur.

Açar sözlər: *Neftin yağ distillatları, efir yağları, ağcaqanadlara təsiri*

Изучение влияния композиции минеральных и эфирных масел как отпугивающего комаров фактора

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Целью нашего исследования являлась подготовка и разработка эффективных фитопрепаратов в качестве средств защиты от комаров, оказывающих сильное отпугивающее действие в местных условиях. Поскольку уникальные масла Азербайджана богаты нафтеновыми углеводородами, они подходят для получения косметических и лечебных масел. Нами получены косметические масла путем удаления жирных дистиллятов из ароматических углеводородов с участием природных и синтетических адсорбентов и изучен их состав. Несмотря на то что при растирании рук очищенными дистиллятами масел в контрольном варианте никаких результатов получено не было, положительные результаты были получены в варианте с изученными эфирными маслами и их смесями.

Ключевые слова: *Дистилляты масла нефти, эфирные масла, воздействие на комаров*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 151-158 (2020)

Influence of football activities on the amount of lipid peroxidation products and enzyme activity in adolescent saliva

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Accepted for publication: 15 May 2020

The article concerns the study of the role of regular physical activity in adaptive changes in the lipid peroxidation process and the antioxidant reaction of the organism in 10–15-year-old adolescents involved in football. Measurements and analysis of lipid peroxidation products (heptan- and isopropanol-soluble products) were performed in the saliva of athletes in various age groups|: 10-11, 12-13 and 14-15 years old. The obtained data indicate that the biochemical analysis of saliva can characterize not only the level of training loads received by the athlete, but also assess the reserve possibilities of adaptation to future loads, as evidenced by the indicators of the enzymatic activity of saliva measured for the antioxidant enzyme catalase and the metabolic enzyme α-amylase. Determining the nature of adaptive changes in indicators of lipid peroxidation in saliva in response to physical exercise will be of significant practical importance, especially, in light of the demand for modern functional and laboratory diagnostics methods that allow fast and effective non-invasive, painless testing.

Keywords: *Adolescent football players, physical training loads, saliva, lipid peroxidation, enzyme activity*

INTRODUCTION

As with some sports, the constant increase in training loads in football leads to a number of functional and biochemical changes of an adaptive nature, which cause improving both of working capacity and effectiveness of recovery processes at different stages of training. It should be noted that the most important task in the preparation of athletes is the gradual intensification of the training process, i.e. a consistent increase in the intensity of training loads. In the practice of sports, an effective increase in the intensity of training loads is achieved on the basis of an individual approach to each athlete. However, physical loads used in football training can be highly effective only when they are scientifically justified, and are implemented in a certain sequence, taking into account the functional capabilities of the organism, recovery potential and diet (Михайлов**,** 2016; Əliyev və b., 2018a). Nutrition and oxygen requirements of the organism must meet its energetic and plastic needs. The main negative effect of physical activity is considered to be a discrepancy between the amount of oxygen entering the body from the external environment and its use in mitochondria for energy production, which leads to the formation of oxidative stress.

Under the influence of oxidative stress, free radical oxidation of lipids (lipid peroxidation - LPO) develops in cells. In normal physiological conditions, this process is controlled by regulatory antioxidant systems (Baraboy, 1989; Alessio, 1993; Əliyev və b., 2018b). Excessive development of free-radical reactions in tissues and cells is opposed by a special antioxidant system consisting of various enzymes and low-molecular compounds. Normally, this system is able to prevent excessive growth of the LPO and maintains its rate at a certain level characteristic of a particular tissue. However, with significant activation of peroxide processes in the body, antioxidant protection is ineffective and free radical oxidation has a pronounced damaging effect on the membranes, thereby causing a certain pathology. However, within the "capacity" of antioxidant protection, the level of

doi.org/10.29228/jlsb.54

LPO processes can vary in a certain interval and characterize urgent metabolic features of a functioning organism (Владимиров, 2000).

For muscular activity, an increase in the level of LPO above the endogenous level of rest occurs during physical exertion, which seems to be a functional feature of the motor system. Thus, it is shown that the level of LPO products in blood plasma and erythrocytes membranes is an important indicator of the adaptive processes of metabolic adjustment in the organism of athletes under the influence of intense physical activity in basketball players (Луцик и др., 2001). Moreover, there is evidence that the specifics of the training and competition process in skiing leads to an optimal intensification of the LPO, in which the content of lipoperoxides increases to values that optimize aerobic metabolism (Дятлов и др., 1997).

The relationship between the two types of action of LPO processes, namely the action that leads to the pathology of the functions of cellular structures and the action that optimizes cellular processes to perform an increased function, is a special subject of free radical biology. At the same time, for the biochemistry of sports, these issues are of crucial importance; almost any sports activity is accompanied by the activation of peroxide reactions. Therefore, research in this area should be aimed at identifying patterns of LPO development, depending on both the nature of the physical work performed, and the orientation of training processes.

Taking into account the fact that at various physical exertions we will face either the pathological effect of LPO, or its “improving” effect, it is necessary to clarify both the possibility of preventing free-radical reactions by using exogenous antioxidants, and the use of LPO indicators (in minimally invasive or non-invasive objects, for example, in blood or saliva) as a criterion for an objective assessment of the athlete’s special training level.

An equally important reason for the activation of LPO processes is the stressful nature of physical activity in modern sports. According to many researchers, excessive activation of the sympathoadrenal system is one of the main causes of structural and functional changes in organs and systems during extreme physical exertion (Meerson, 1985; Дятлов и др., 1997; Григорьева, 2003). The adaptive lipotropic effect of the stress reaction can also turn into a damaging effect due to increased free radical production and subsequent activation of free radical oxidation (Львовская, 1998; Baraboy, 2006; Стаценко и Алькевич, 2009). It should be taken into account that the organism's systems, including the antioxidant defense system, adapt not to physical activity in general, but to a specific type of muscle activity. The orientation of the training process significantly affects the state of the oxidant-antioxidant system, since adaptive changes in metabolism that are characteristic of different sports are specific. Specific metabolic changes are formed in the body of athletes, which are manifested both at rest and in response to physical activity (Львовская, 1998**;** Baraboy, 2006; Базагин, 2013).

The effect of stress on the human organism leads to the activation of the sympathetic nervous system (SNS), which innervates all organs. Activation of SNS is correlated with changes in the activity of α-amylase in saliva (van Stegeren et al., 2006). So monitoring the activity of this enzyme in the saliva of athletes is of considerable interest in terms of reaction to the effects of physical activity.

Based on the above, we have devoted this study to studying the role of regular physical activity in adaptive changes in the processes of lipid peroxidation, antioxidant and metabolic reactions in adolescent footballers, studying the corresponding indicators in saliva.

MATERIALS AND METHODS

The work with athletes was carried out in accordance with the standards of training of young athletes (Nabatnikova, 1984**).**

Saliva was used for biochemical analyzes. Before taking a saliva sample, the oral cavity was rinsed with saline solution, and then about 2 ml of saliva was collected in test tubes for 35 minutes. Saliva samples were centrifuged at 3000 rpm for 15 minutes. Supernatant was used for further research. The LPO level was assessed by the content of primary (diene conjugates of hydroperoxides) and secondary (ketodienes and conjugated trienes) products of LPO in heptan-isopropanol extracts of saliva. Determination of LPO products in hepta-

ne-isopropanol extracts of saliva was performed by the spectrophotometric method according to I.A.Volchegorsky et al. (Волчегорский и др., 1989). The results were calculated in the form of oxidation indices-E232/E220 and E278/E220, which reflect the relative level of primary (diene conjugates) and secondary (ketodienes and conjugated trienes) LPO products, respectively.

The lipid fraction of 0.5 ml samples was extracted in 5 ml of a mixture of equal volumes of heptane and isopropanol by shaking for 15 minutes. The lipid extract was separated by centrifugation and diluted with 5 ml of the heptane-isopropanol mixture (3:7 by volume) and divided into phases by adding 2 ml of an aqueous HCl solution (pH=2). After 30 minutes, the heptane (upper) phase was transferred to a separate test tube, and 1 g of dry sodium chloride was added to the water-alcohol (lower) phase to separate the water phase. After that, they were shaken again for 5 minutes. After 20 minutes, the obtained extracts were selected and measured at the appropriate wavelengths of the ultraviolet range.

Determination of catalase activity was performed by a method based on the reaction of hydrogen peroxide with catalase and determination of light absorption of a complex of hydrogen peroxide with ammonium molybdate at a wavelength of λ=410 nm. For this purpose, 25 ml of drinking water was mixed with 2 ml of 0.03% hydrogen peroxide. The reaction was stopped by adding 2 ml of 2% ammonium molybdate after 10 minutes. In parallel, control experiments were conducted without the participation of enzyme. The difference in the optical densities of the control and experimental samples was used to calculate the enzyme activity (molar absorption coefficient - 22.2×10^3 $cm^{-1}M^{-1}$).

The activity of α-amylase was determined by the test using 2-chloro-4-nitrophenyl-maltotrioside (CNPG3) as a substrate. The reaction is catalyzed directly by α-amylase. The formation of 2-chloro-4-nitrophenol (CNP) leads to an increase in optical density over time. The activity was determined by the rate of accumulation of CNP, which is proportional to enzyme concentration in sample. Saliva samples were diluted 200 times.

SPSS for Windows version 22.0 package program was used for statistical analyses of data. Shapiro–Wilk test was used to check whether the variables for studied groups fit normal distribution. Differences between control and experimental measurements were tested using paired samples t-test. Mean ± standard error values were given as the descriptive statistics and $p<0.05$ was accepted as the statistically significant value.

RESULTS AND DISCUSSION

The table illustrates data on the age dynamics of saliva biochemical parameters in adolescent football players in comparison with adolescents who do not engage in active sports. Biochemical indicators are products of lipid peroxidation, namely, the concentration of neutral lipids heptan_1, heptan_2, as well as phospholipids isopropanol_1, isopropanol_2. In addition, the activities of the antioxidant enzyme catalase and the amylase enzyme in saliva of adolescent football players of the same age groups of 10-11, 12-13 and 14-15 years are shown in the table. The analysis of these indicators characterizes the functioning of antioxidant factors of non-specific protection and the state of LPO in organism of young athletes.

The analysis of LPO indicators in young football players shows that the content of heptan-soluble products in saliva does not significantly change in different age groups, which implies independence from the experience of playing football. A similar pattern was observed in the amount of LPO products solubilized in isopropanol. This means that the antioxidant system of non-specific adaptation functions optimally, that is, there is no reliable increase in LPO products under the influence of various physical loads to the organism of adolescents, with no damaging effect on the membrane of cells and tissues.

A comparative analysis of the results of non-invasive biochemical study of the saliva in adolescent athletes and non-athletes of the same first age group showed that heptane-soluble primary and secondary peroxide products of lipids (Heptan_1 and Heptan_2) have significant differences ($p<0.05$) in subjects aged 10-11 years: the excess in adolescent football players is 10.9% for Heptan_1, and 17.9% - for Heptan_2.

Table. Pecularities of biochemical analysis indicators of saliva in adolescent football players and non-sporting adolescents (M±m)

Indicators of biochemical analysis	I CG (n=12)	I EG (n=16)	II CG (n=12)	II EG (n=15)	III CG (n=12)	III EG (n=12)
LPO product Heptane_1	0.172±0.030 100%	0.190±0.016^{+} 100%	0.183 ±0.030 107.2%	0.184±0.020 97.0%	179.0±0.011 105.9%	0.181±±0.011 107.1%
LPO product Heptane_2	0.120±0.095 100%	0.144±0.060^{+} 100%	0.124±0.027 102.4%	0.138±0.035 97.9%	0.128±0.035 105.8%	0.134±0.032 95.1%
LPO Product Isopropanol_1	0.450±0.032 100%	0.560±0.025^{++} 100%	0.514±0.060 115%	0.547±0.016^{+} 99.8%	0.516±0.077 114.7%	0.542±0.022 98.9%
LPO product Isopropanol_2	0.310±0.045 100%	0.311±0.072 100%	0.313±0.024 100%	0.313±0.014 100%	0.325±0.019 104%	0.305±0.35 98%
Catalase. nmol/mg/min	140.0±3.20 100%	138.97± 2.20 100%	144.94± 3.35 103%	147.39±2.50^{++***} 106%	140.45±1.46 102%	153.68± 2.30^{++***} 108%
α-Amylase. nmol/mq/min	455.0±2.60 100%	456.95± 1.65 100%	451.36±2.10 98.5%	462.45±2.27^{++***} 101%	459.99±2.23 100.5%	466.97±2.40^{++***} 102.2%

I CG, I EG - control and experimental groups of 10-11 year old, initial training;
II CG, II EG - control and experimental groups of 12-13 year old, training for 1-2 years;
III CG, III EG - control and experimental groups of 14-15 year old, training for 3-4 years.
+ - $p < 0.05$, ++ - $p < 0.01$ - reliable changes in adolescent athletes compared to control group.
* - $p< 0.05$, *** - $p < 0.001$ - reliable changes to group I.

In other age groups, heptane-soluble lipoperoxide products in saliva do not have significant differences between athletes and non-athletes. This is due to the fact that the products of lipid peroxidation in the organism of young athletes accumulate in response to physical activity at the initial stage of sports training.

The content of isopropanol-soluble primary LPO products (Isopropanol_1) in the saliva of 10-11 year old football players was significantly higher by 24.4% than in the corresponding control group ($p<0.01$). In other age groups of football players, the content of Isopropanol_1 is also more or less significantly higher than the level of the corresponding control groups; in the group of 12-13 years by 6.4% ($p<0.05$), and in the group of 14-15 years – by 5.1% ($p<0.05$). Secondary products of isopropanol-soluble LPO products (Isopropanol_2) did not show any significant changes in all groups of adolescent football players when compared with adolescents who do not engage in active training.

Catalase, an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide formed during biological oxidation into water and molecular oxygen. This enzyme can give rise to more dangerous active forms of oxygen, including a highly reactive hydroxyl radicals that can easily induce oxidative stress in the cell, thereby damaging biological membranes.

The study of catalase activity in saliva of adolescent athletes, who play football and are at different levels of training, showed that 10-11 year olds do not show a significant difference in the activity of the enzyme in relation to the control group of adolescents who are not athletes. Contrary to this, athletes with a long experience of football training have increased catalase activity when compared with control groups. A group of 12-13 year old football players (1-2 years of football training) showed an increased level of catalase activity by 2.2% ($p<0.01$), and a group of 14-15 year old football players (3-4 years of football training) - by 9.3% ($p<0.01$) compared with control persons.

The activity of the α-amylase enzyme as well as catalase does not show significant changes in 10-11 year-olds engaged in football, in relation to control adolescents. At the same time, 12-13 year old football players with 1-2 years of experience have amylase activity in saliva by 2.4% ($p<0.01$) higher than the activity level for non – athletes, and 14-15 year old football players with 3-4 years of experience-by 1.7% ($p<0.01$) of the control level.

An analysis of changes in the activity of catalase, an antioxidant enzyme in adolescent football players revealed that the adaptation reactions to physical loads have a phased nature in functioning of the antioxidant defense system in 10-15 year old players. Reliable increase in activity of the ca-

talase and α-amilase enzymes ($p<0.05$) was observed in 12-13 year-old compared to non-athletes and an increase of about 3.7%. Such a positive growth trend was also observed in adolescents aged 14-15, with an increase of 6.9% (Figure 1 and Figure 2).

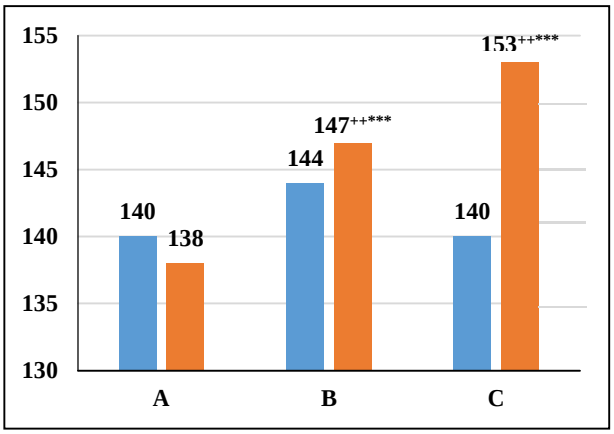

Fig. 1. Catalase activity (nmol/mg/min) in saliva of adolescent football players and non-sporting adolescents. A - I group, 10 - 11 year old; B - II group, 12 - 13 year old; C - III group, 14 - 15 year old. Blue - control, red - athletes. *** - $p<0.001$ - compared to I group athletes; ++ - $p<0.01$ - compared to control group.

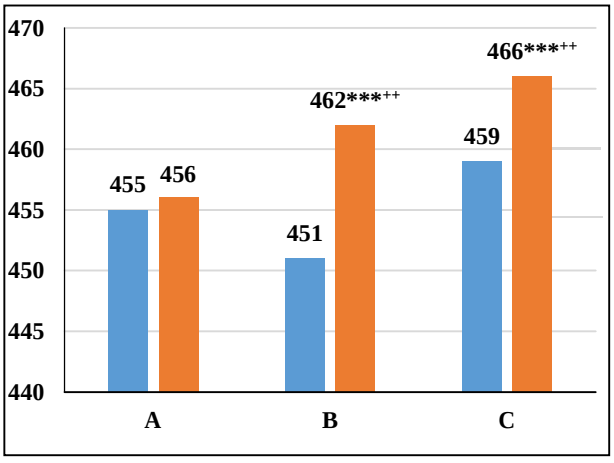

Fig. 2. α-Amylase enzyme activity (nmol/mg/min) in saliva of adolescent football players and non-sporting adolescents. All designations as in Figure 1.

The biochemical adaptation of organism to the muscle activity during the course of the training process extends to all functional systems related to the physical activity. Of course, this effect does not overlook the antioxidant system. The basic chemical mechanisms of adaptation changes in these systems and organs are identical to the biochemistry of adaptation changes that occur in the muscles (Meerson, 1985; Михайлов, 2016).

Strengthened energy processes in the body under the influence of intensified physical loads increase the amount of oxygen consumption by the organism, and its transport from the cell membrane is intensified. At this point, part of the oxygen transported is oxidized by contacting the membrane's structural compounds. As a result, particles or radicals, called free radicals, form on the cell membrane. As these particles have unused energy and unconnected electrons, they increase their ability to react highly, removing electrons from adjacent particles, complementing their outer electron shell, and converting the substance into free radical carrier. As a result, free radicals stimulates the chain development. It should also be noted that under normal physiological conditions, free-radical oxidation of lipids in the cells occurs partly and is under the direct control of these regulatory systems (Alessio, 1993; Григорьева, 2003). Moderate doses of free radicals are involved in the regulation of biological membrane functions and the renewal of their chemical composition by LPO products (peroxide oxidation of lipids) of biological membranes. It is well known that activation of sympatoadrenal and hypothalamic - hypophysis - renal systems under the influence of external factors (including physical loads) stimulates increased LPO levels (Meerson, 1985; Григорьева, 2003; Михайлов, 2016)

The stress reactions that occur in response to physical stress are accompanied by the activation of various stress-limiting reactions. Stress-limiting metabolites include classical hormones, neuromediators, and various enzymes (superoxidismutase, catalase, glutathione peroxidase, α-amylase ets.) (Stoney, 1997; Еликов и др., 2017**)**.

Our results indicate that the biochemical analysis of saliva can characterize not only the level of training loads received by the athlete, but also assess the reserve possibilities of adaptation to future loads, as evidenced by the indicators of the enzymatic activity of both antioxidant and metabolic nature.

CONCLUSION

Thus, the analysis of the biochemical parameters of saliva in adolescent soccer players showed that the amount of first and second products of peroxide oxidation of lipids were not significantly reduced. This also indicates that the response to anxious reactions under the influence of physical exercise loads has been more economical. More precisely, the performance of LPO products is more important than the effect of different physical loads. In addition, increases in the activity of the antioxidant enzyme catalase and the α-amylase enzyme confirms the increased antioxidant component of non-specific protection in the organism of adolescents. Positive adaptive changes result from the stress-damaging effects of physical loads. It should be noted that the positive changes in the training process with the players are aimed to adapting the antioxidant system to their physical activity.

Determining the nature of adaptive changes in indicators of lipid peroxidation in saliva in response to physical exercise will be of significant practical importance, especially, in light of the demand for modern functional and laboratory diagnostics methods that allow fast and effective non-invasive, painless testing.

REFERENCES

Əliyev S.A., Əliyev İ.S, Əlibəyova S.S. (2018a) Əzələ fəaliyyətinə 13-15 yaşlı futbolçuların orqanizminin fizoloji sistemlərinin adaptasiyası. *Biologiyanın müasir problemləri. Respublika elmi konfransının materialları*. Sumqayıt: 294-295.

Əliyev S.A., Həsənova A.K., Əhmədova İ.N. (2018b) Antioksidant müdafiə sistem fermentlərinin aktivliyinin idmançıların qan plazmasında dəyişilməsi dinamikası. *AMEA-nın A.İ.Qarayev adına Fiziologiya İnstitutu və Azərbaycan Fiziologlar Cəmiyyətin Elmi əsərlərinin külliyatı. Fizologiyanın və biokimyanın problemləri*, **36:** 113-119.

Базагин К.П. (2013) Динамика показателей антиоксидантного статуса у спортсменов. *Бюл. ВСНЦСО*, **5:** 9-12.

Владимиров Ю.А. (2000) Свободные радикалы в биологических мембранах. *Соросовский образовательный журнал*, **6(12):** 13-19.

Волчегорский И.А., Налимов А.Г., Яровинский Б.Г. и др. (1989) Сопоставление различных подходов к определению продуктов перекисного окисления липидов в гептан-изопропанольных экстрактах крови. *Вопросы медицинской химии*, **35(1):** 127-131.

Григорьева Н.М. (2003) Особенности адаптации системы перекисного окисления липидов и антиоксидантной системы (ПОЛ-АОС) к регулярным занятиям плаванием у детей 7-9 лет. *Дис. к.б.н.* Челябинск: 154 с.

Дятлов Д.А., Волчегорский И.А., Львовская Е.И. и др. (1997) Анализ содержания продуктов липопероксидации в крови лыжников-гонщиков различной спортивной квалификации. *Теория и практика физической культуры*, **4:** 59-63

Еликов А.В., Галстян А.Г. (2017) Антиоксидантный статус у спортсменов при выполнении дозированной физической нагрузки и в восстановительном периоде. Вопросы питания. **86(2):** 23-3

Львовская Е.И. (1998) Нарушение процессов липидной пероксидации при термической травме и патогенетическое обоснование лечения антиоксидантами из плазмы крови: *Дис. … д-ра мед. Наук*. Челябинск: 260 с.

Луцик Е.Г., Коношенко С.В., Попичев М.И. (2001) Состояние перекисного окисления липидов плазмы крови и эритроцитарных мембран у баскетболистов. Физиология человека. **27(6):** 108-110

Михайлов С.С. (2016) Биохимия двигательной деятельности. Москва: Советский спорт, 296 с.

Стаценко, Е.А., Алькевич Е.Л. (2009) Окислительный стресс в дозировании физи-ческих нагрузок. *Физиотерапия, бальнеоло-гия и реабилитация*, **№4:** 33–36.

Alessio H.M. (1993) Exercise-induced oxidative stress. *Med. Sci. Sport Ex*ers., **25(2):** 218-224.

Baraboy V.A. (1989) The role of lipid peroxidation in the mechanism of stress. *Fiziologicheskiĭ zhurnal*, **35(5):** 85-97.

Baraboy V.A. (2006) Bioantioxidants. Kiev: Book Plus Publ., pp. 154–155.

Meerson F.Z. (1985) Physiology of adaptation processes. M: Nauka, 645 p. (in Russian).

Metzler D.E. (1980) Biochemistry: The chemical reactions of living cells. Edited by A.E. Brownstein. M: Mir, **1(3):** 50-60.

Nabatnikova M.Y. (1984) Methodology for constructing long-term norms of versatile physical and functional training of young athletes. M: Physical culture and sport, 84 p. (in Russian).

Sen C.K. (1995) Oxidants and antioxidants in exercise. *J. Appl. Physiol*., **79(3):** 675-686.

Stoney C.M., Bausserman L. Niaura R. et al. (1999). Lipid reactivity to stress: II. Biological and behavioral influences. Health Psychology, **18(3):** 251–261. https://doi.org/10.1037/0278-6133.18.3.251

Van Stegeren A., Rohleder N., Everaerd W., Wolf O.T. (2006) Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*, **31(1):**137-41.

Yamamoto T., Ohkuwa T., Itoh H. et al. (2003) Relation between voluntary physical activity and oxidant/antioxidant status in rats. *Comparative Biochemistry and Physiology. Part Toxicology and Pharmacology*, 1**35(2):** 163-168.

Futbol məşğələlərinin yeniyetmələrin ağız suyunda lipidlərin peroksidləşmə məhsullarının miqdarına və fermentativ aktivliyə təsiri

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Məqalə futbol idmanı ilə məşğul olan 10-15 yaşlı yeniyetmələrdə lipidlərin peroksidləşməsi proseslərinin adaptasiya dəyişikliklərində müntəzəm fiziki yüklənmələrin rolunun və orqanizmin antioksidant reaksiyasının öyrənilməsinə həsr olunmuşdur. Lipidlərin peroksidləşmə məhsullarının (heptanda və izopropanolda həll olunan məhsulların) ölçülməsi və təhlili 10-11, 12-13 və 14-15 yaşlı yeniyetmələrdən ibarət qrupları üzrə idmançıların ağız suyunda aparılmışdır. Alınan məlumatlar göstərir ki, ağız suyunun biokimyəvi analizi yalnız idmançının əldə etdiyi məşq yüklərinin səviyyəsini xarakterizə etməyə deyil, həm də gələcək yüklənmələrə adaptasiyanın ehtiyat imkanlarını qiymətləndirməyə imkan verir. Fiziki yüklənməyə cavab olaraq ağız suyunda lipidlərin peroksidləşmə göstəricilərində adaptiv dəyişikliklərin xarakterinin müəyyən edilməsinin, bugün qeyri-invaziv, ağrısız testlər aparılmasını operativ və effektiv həyata keçirməyə imkan verən funksional və laborator diaqnostikanın müasir metodlarına yüksək tələbatın olması baxımından müəyyən praktik (diaqnostik) əhəmiyyətə malik olması şübhə doğurmur.

Açar sözlər: *Yeniyetmə futbolçular, fiziki məşq yükləri, ağız suyu, lipid peroksidləşməsi, fermentativ aktivlik*

Влияние футбольных тренировок на уровень продуктов перекисного окисления липидов и ферментативную активность в слюне подростков

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Работа посвящена исследованию роли регулярных физических нагрузок в адаптивных изменениях процесса перекисного окисления липидов и антиоксидантной реакции организма у 10-15 летних подростков, занимающихся футболом. Были проведены измерения и анализ продуктов перекисного окисления липидов в слюне спортсменов в различных возрастных группах 10-11, 12-13 и 14-

15 лет. Полученные данные указывают на то, что биохимический анализ слюны может характеризовать не только уровень тренировочных нагрузок, получаемых спортсменом, но и оценить резервные возможности адаптации к будущим нагрузкам, о чем свидетельствуют показатели ферментативной активности слюны, измеренной для антиоксидантного фермента каталазы и метаболического фермента α-амилазы. Установление характера адаптивных изменений показателей ПОЛ в слюне в ответ на физические нагрузки имеет практическое (диагностическое) значение, особенно, в свете востребованности в современной функциональной и лабораторной диагностике методов, позволяющих быстро и эффективно проводить неинвазивные, безболезненные тестирования.

Ключевые слова: *Футболисты подросткового возраста, физические тренировочные нагрузки, слюна, перекисное окисление липидов, ферментативная активность*

Journal of Life Sciences & Biomedicine, vol. 2(75), No 1, p. 159-166 (2020)

Soft tissues regional blood flow and microvasculature upon different perioperative treatment strategies following indirect revascularization in patients with critical lower limb ischemia, caused by occlusion of distal arteries

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Accepted for publication: 19 June 2020

This work was aimed at studying the effect of different perioperative treatment strategies following indirect revascularization on regional blood flow and microcirculatory network of soft tissues in patients with critical lower limb ischemia. The study was conducted in 210 patients (154 men, 56 women) with critical lower limb ischemia aged from 28 to 74 years, who were receiving hospital treatment during 2001-2019, with the ischemia duration of two-months-up-to-four-years. The cause of critical ischemia was the occlusion of the arteries of the femoral-popliteal-tibia and tibia-foot segments of the atherosclerotic and thromboangiotic genesis. The regional blood flow was examined through non-invasive instrumental techniques (the determination of oxygen saturation of skin, rheovasography, Doppler ultrasonography, ultrasound duplex scanning). The state of the microvasculature and neoangiogenesis was evaluated by complex morphological and immunohistochemical studies of tru-cut biopsy specimens of ischemic limb soft tissues in 80 patients (55 men, 25 women). Non-parametric tests were used to analyze digital data. It was found that in contrast to controls, the combined administration of intravenous laser blood irradiation and cytokine therapy with 'Roncoleukin' in the perioperative period of revascularizing osteotrepanation with intraosseous laser irradiation accelerates neoangiogenesis and increases the number of functionally active microvessels with open lumen in the soft tissues of ischemic limb. The statistically significant improvement in regional blood flow and skin oxygen saturation was observed after indirect revascularization as well. The combination of intravenous laser blood irradiation and cytokine therapy in the perioperative period of indirect revascularization by osteotrepanation with bone marrow laser irradiation creates favorable conditions for increasing regional blood flow and promoting neoangiogenesis to improve microvasculature in critically ischemic lower limb.

Keywords: *Occlusion of arteries, critical ischemia, indirect revascularization, laser irradiation, cytokine therapy, regional hemodynamics, microvasculature*

INTRODUCTION

The treatment and prognosis of the critical lower limb ischemia (CLLI), caused by the occlusion of their distal arteries have not lost their relevance. Reconstructive surgery of the arteries of lower extremities in CLLI is the main treatment strategy for this severe category of patients; however, in 5.8-16.5% of cases there are adverse outcomes, and in 8.8-20.4% – major amputations (Бокерия и соавт, 2011; Буров и соавт., 2019; Затевахин и соавт., 2011; Казаков и Жук, 2019; Покровский и соавт., 2018; Нац. рекомендации, 2019; Biancari et al., 2007). X-ray endovascular surgery does not always provide a complete revascularization of the limb (Затевахин и соавт., 2011; Казаков и Жук, 2019; Ховалкин и соавт., 2019; Bosh et al., 2007; Clair et al., 2005). Conservative therapy as an alternative to disabling surgery (disabling sur-

doi.org/10.29228/jlsb.55

gical intervention) is also ineffective (Нац. Рекомендации,2019). In the surgical treatment of patients with CLLI, a separate group is made up of indirect methods of revascularization which includes revascularizing osteotrepanation (ROT), lumbar sympathectomy (LSC) in combination with other surgical interventions and various modifications of perioperative complex therapy (Гасанов и Косаев, 2019; Зусманович, 1999; Косаев, 2011; 2012а; 2012б; Кротовский и Зудин, 2005; Суховатых и Орлова, 2013; Aslyaev et al., 2016). The state of the microvasculature of the affected soft tissues of the lower extremities of patients with CLLI under different schemes of perioperative treatment with indirect revascularization remains poorly understood, especially from the standpoint of modern morphology and non-invasive instrumental diagnostics (Nowak-Sliwinska et al., 2018). Meanwhile, an objective assessment of the morphological and functional state of micro-vasculature would not only optimize perioperative therapy in indirect revascularization, but also improve the prognosis in patients with CLLI.

OBJECTIVES

The purpose of this research is a comprehensive non-invasive-instrumental and morphological study of regional blood flow and microvasculature of soft tissues under various perioperative treatment regimens following indirect revascularization in patients with critical lower limb ischemia (CLLI) caused by the occlusion of their distal arteries.

MATERIALS AND METHODS

A permission to conduct the study was obtained from the Ethics Committee of the Scientific Center of Surgery named after Acad. M.A.Topchubashev. The study contingent was composed of 80 patients who were familiarized with all aspects of the upcoming treatment before it began and gave written consent to carry it out.

Twenty-eight-to-seventy-four years old patients of both sexes (55 men and 25 women) suffering from critical ischemia from 2 months up to 4 years were hospitalized in the Department of Vascular Surgery of the Scientific Center of Surgery named after Acad. M.A.Topchubashev in the period from 2001 to 2019. The reason for the development of critical ischemia was the unreconstructable occlusion of femoral-popliteal-tibial and tibial-foot segments of arteries, which was of atherosclerotic and thromboangiotic origins. As concomitant diseases, there were arterial hypertension, coronary heart disease, chronic cerebrovascular disease, chronic obstructive bronchopulmonary diseases, as well as pyloroduodenal erosive lesion and renal failure.

In order to establish a diagnosis of critical lower limb ischemia and evaluate the effectiveness of the proposed method, clinical and instrumental studies were carried out: the determination of oxygen saturation of skin, rheovasography, Doppler ultrasonography, ultrasound duplex scanning, and multi-spiral computed tomography angiography. Using instrumental methods of research, we determined such indicators of arterial and venous blood flow as the rheographic index (RI), linear velocity of blood (LVC) in the popliteal artery, gradient of regional systolic pressure (GRSP), gradient of post-occlusive venous pressure (GPOVP), venous arterial index (VAI), which was measured in the standing and supine positions. Oxygen saturation of skin in the distal part of the foot was also studied.

In the perioperative period, 34 patients received standard treatment, 32 patients - standard treatment plus intravenous laser blood irradiation, 32 patients - standard treatment plus cytokine therapy with ‘Roncoleukin’, 33 patients - standard treatment plus intravenous laser irradiation of blood along with the cytokine therapy with the 'Roncoleukin', 31 patients - in the ROT with intraosseous laser irradiation - standard treatment plus intravenous laser blood irradiation along with the cytokine therapy with «Roncoleukin».

The state of regional arterial and venous blood flow in patients was studied upon their admission to the clinic and upon the completion of treatment. Some of the parameters of regional arterial and venous blood circulation were compared to the identical indicators of 48 healthy individuals (the “reference group”).

The microvasculature of ischemic soft tissues of the lower extremities was studied in a total of 80 patients (55 men, 25 women). It was performed by non-traumatic tru-cut biopsy of soft tissu-

es of the foot (skin, subcutaneous tissue, fascio-aponeural layer and muscles), lower leg and thigh, along with general histological (hematoxylin-eosin, picrofuxin) and immunohistochemical analyzes **(**VEGF, CD31, CD34, collagen of IV type and Ki67; "Roche Diagnostics") according to the standard procedure (Dey, 2018).

To determine the possible correlations of the studied parameters with various schemes of perioperative treatment, the obtained clinical and instrumental data was processed by using non-parametric tests with the calculation of the Pearson's chi-squared test ($\chi 2$) and correlation coefficient (r) at a confidence level of P = 0.95 (Юнкеров В.И. и соавт., 2011).

RESULTS AND DISCUSSION

Regional arterial and venous blood flow was characterized by controversial changes. Upon admission to the clinic, patients had severe blood flow disturbances, which were also accompanied by a significant suppression of the level oxygen saturation of skin. Revascularizing osteotrepanation, in general, had a positive effect on the studied parameters of regional blood flow, which was confirmed by an increase in the number of patients with positive clinical dynamics. Moreover, the best results were observed for operations of revascularizing osteotrepanation with simultaneous laser irradiation of bone marrow in the postoperative period during 7-8 days. It is pertinent to mention that upon completion of treatment, 25-27 out of 31 patients showed a significant improvement in almost all the considered indicators of regional blood flow in the critically ischemic limb. Of particular note are the rheographic index and the gradient of regional systolic pressure, the improvement of which was recorded in 87.1% of patients (Table 1).

In parallel with instrumental non-invasive examination of regional blood circulation, neoangiogenesis and the density of microvasculature in the small soft tissue biopsy specimens of critically ischemic limb were also comprehensively studied based on a combination of a number of morphological parameters. In particular, neoangiogenesis analysis revealed that after the completion of complex treatment, 40 patients out of the examined 80 (50.0%) showed an acceleration of the formation of new microvessels in previously necrobiotic tissues, especially in the endomysium of muscles and fascial-adipose tissue. The maximum stimulation of this process was inherent to the subgroup of patients who received intraosseous and intravenous laser irradiation in combination with cytokine therapy in the perioperative period (14 patients with an increase of neoangiogenesis; 77.8%; Table 2).

The above-mentioned data allowed us to suggest that laser stimulation of the bone marrow and venous lining in combination with immunomodulatory therapy in the perioperative period can promote the formation of microvessels, which is consonant with modern understanding of angiogenesis (Nowak-Sliwinska et al., 2018).

An analysis of the microvasculature density indices upon completion of treatment of patients revealed a similar pattern. Thus, the total density of various microvessels (arterioles, precapillaries, hemocapillaries and postcapillary venules) tended to increase in 34 patients out of 80 examined (42.5%). As in the case of neoangiogenesis, the maximum increase in the average number of microvessels was detected in patients with intraosseous and intravenous laser irradiation in combination with cytokine therapy in the perioperative period (11 patients; 61.1%; Table 3).

It should be noted that the morphologically verified microvessels of a specific necrobiotic-altered (but not necrotic) tissue microzone were roughly divided into two types: functional (with preserved structure and lumen) and non-functional (without a clearly structured wall and lumen less than 4.1 μm). Based on the results, the proportion of functional microvessels during perioperative therapy accompanying the revascularizing osteotrepanation with bone marrow laser irradiation was higher in those patients who underwent complex perioperative treatment with intravenous laser irradiation in combination with cytokine therapy.

Table 1. The indicators of regional blood flow and oxygen saturation of skin under the usage of various perioperative therapy strategies for indirect revascularization in patients with critical ischemia of the lower extremities of distal arterial occlusive origin (number of patients, $\chi 2$; p; r *)

Indicators	Study groups	Cont. group n=48	ROT operation n=42		LST operation n=51		ROT operation + LST n=38		ROT operation with BMLI n=31	
VAI lying	decreased	18	25	χ^2=4,355 p<0,05 r=0,3	32	χ^2=6,304 p<0,05 r=0,4	28	χ^2=11,162 p<0,001 r=0,5	26	χ^2=16,414 p<0,001 r=0,6
	not changed	30	17		19		10		5	
VAI standing	decreased	17	25	^{2}=5,230 p <0,05 r=0,3	34	χ^2=9.668 p <0,01 r=0,4	28	χ^2=12,451 p <0,001 r=0,5	25	χ^2=15,474 p <0,001 r=0,6
	not changed	31	17		17		10		6	
GRSP	decreased	21	28	χ^2 =4,473 p <0,05 r = 0,3	33	χ^2 =4,380 p <0,05 r = 0,3	27	χ^2 =6,411 p <0,05 r = 0,4	27	χ^2 =14,844 p <0,001 r = 0,6
	not changed	27	14		18		11		4	
GPOVP	decreased	19	26	χ^2 =4,464 p <0,05 r = 0,3	31	χ^2 =4,446 p <0,05 r = 0,3	29	χ^2 =11,604 p <0,001 r = 0,5	26	χ^2 =15,070 p <0,001 r = 0,6
	not changed	29	16		20		9		5	
RI	increased	20	27	χ^2=4,593 p<0,05 r=0,3	36	χ^2=8,418 p<0,05 r=0,4	28	χ^2=8,816 p<0,01 r=0,4	27	χ^2=16,131 p<0,001 r=0,6
	not changed	28	15		15		10		4	
LVC	increased	18	26	χ^2=5,339 p <0,05 r=0,3	32	χ^2=6.304 p <0,001 r=0,4	28	χ^2=11,162 p <0,001 r=0,5	26	χ^2=16,414 p <0,001 r=0,6
	not changed	30	16		19		10		5	
OSS	increased	18	25	χ^2 =4,355 p <0,05 r = 0,3	33	χ^2 =7,328 p <0,01 r = 0,4	29	χ^2 =12,894 p <0,001 r = 0,5	26	χ^2 =16,414 p <0,001 r = 0,6
	not changed	30	17		18		9		5	

Note: ROT - revascularizing osteotrepanation; LST - lumbar sympathectomy; ROT + LST - revascularizing osteotrepanation + lumbar sympathectomy; ROT with BMLI - revascularizing osteotrepanation with bone marrow laser irradiation; VAI - venous arterial index; GRSP - gradient of regional systolic pressure, GPOVP - gradient of postocclusal venous pressure; RI - reographic index; LBV - linear blood velocity; OSS - oxygen saturation of skin;

*- χ^2, p, r were calculated between the corresponding indicators of the control and individual surgical groups of patients

Table 2. The intensity of neoangiogenesis under the conditions of applying various perioperative therapy regimens for revascularizing osteotrepanation in patients with critical lower limb ischemia of distal arterial occlusive origin (at the end of treatment; number of patients, $\chi 2$; p; r*)

Group of Patients	**Indicator**	**The intensity of neoangiogenesis**	χ^2 p r
Comparison (control; n=15)	increased (↑)	5	
	not changed	10	
Intravenous laser irradiation (n=15)	increased (↑)	9	χ^2=2,143 p>0,05 r=0,4
	not changed	6	
Cytokine therapy with 'Roncoleukin' (n=15)	increased (↑)	8	χ^2=1,222 p>0,05 r=0,3
	not changed	7	
Intravenous laser irradiation + cytokine therapy with 'Roncoleukin' (n=17)	increased (↑)	11	χ^2=3,137 p>0,05 r=0,4
	not changed	6	
Bone marrow laser irradiation + intravenous laser irradiation + cytokine therapy with 'Roncoleukin' n=18)	increased (↑)	14	χ^2=6,617 p<0,05 r=0,6
	not changed	4	

Note: * - $\chi 2$, p, r - were calculated between the corresponding indicators of the examined groups of patients

Table 3. The density of microvessels under the conditions of applying various schemes of perioperative therapy for indirect surgical revascularization in patients with critical ischemia of the lower extremities of distal arterial occlusive origin (at the end of treatment; number of patients, $\chi 2$; p; r *)

Group of patients	**Indicator**	**Density of microvessels**	χ^2 p r
Comparison (control; n=15)	increased (↑)	4	
	not changed	11	
Intravenous Laser Irradiation (n=15)	increased (↑)	8	χ^2=2,222 p>0,05 r=0,4
	not changed	7	
Cytokine Therapy with 'Roncoleukinum' (n=15)	increased (↑)	7	χ^2=1,292 p>0,05 r=0,3
	not changed	8	
Intravenous laser irradiation + cytokine therapy with 'Roncoleukinum' (n=17)	increased (↑)	10	χ^2=3,348 p>0,05 r=0,4
	not changed	7	
Bone marrow laser irradiation + intravenous laser irradiation + cytokine therapy with 'Roncoleukinum' (n=18)	increased (↑)	11	χ^2=3,915 p<0,05 r=0,5
	not changed	7	

Note: * - $\chi 2$, p, r - were calculated between the corresponding indicators of the examined groups of patients

CONCLUSIONS

1. The combination of generally accepted conservative treatment regimens (protocols) with bone marrow and intravenous laser irradiation in the perioperative period during revascularizing osteotrepanation operations contributes to the partial repair of the microvasculature, as evidenced by the results of the instrumental and morphological examinations of the regional blood circulation and microcirculation in patients with critical low limb ischemia caused by distal arterial occlusion.
2. Intravenous laser irradiation in combination with cytokine therapy in the perioperative period of revascularizing osteotrepanation with bone marrow laser irradiation produced comparatively better long-term outcomes in correction of the regional blood flow and neoangiogenesis in patients with critical lower limb ischemia caused by distal arterial occlusion.

REFERENCES

Бокерия Л.А., Темрезов М.Б., Коваленко В.И. и др. (2011) Актуальные проблемы хирургического лечения больных с критической ишемией нижних конечностей – пути решения (состояние проблемы). *Анналы хирургии*, **№1:** 5-9.

Буров А.Ю., Артемьева К.А., Азарьян А.С. и др. (2019) Дифференцированный подход в лечении больных с критической ишемией нижних конечностей при атеросклеротическом поражении артерий бедренно-берцового сегмента. *Ангиология и сосудистая хирургия (приложение)*, **25(2):** 81-82.

Гасанов И.А., Косаев Дж.В. (2019) Сочетанное применение внутривенной лазерной терапии, внутрикостного лазерного облучения и ронколейкина при непрямой реваскуляризации у больных дистальным поражением артерий с критической ишемией нижних конечностей: *Материалы научно-практической конференции с международным участием. Москва: Лазерная медицина*, **23(вып. 3S):**16

Затевахин И.И., Шиповский В.Н., Золкин В.Н., Богомазов И.Ю. (2011) Отдаленные результаты открытых эндоваскулярных операций в коррекции окклюзионно-стенотических поражений артерий бедренно-подколенного сегмента. *Ангиология и сосудистая хирургия*, **17(№3):** 59-64

Казаков Ю.И., Жук Д.В. (2019) Сравнительные результаты открытых и эндоваскулярных вмешательств у больных с окклюзией бедренно-подколенного артериального сегмента в стадии критической ишемии нижних конечностей. *Ангиология и сосудистая хирургия (приложение)*, **25(2):** 181-182.

Покровский А.В., Казаков Ю.И., Лукин И.В. (2018) Критическая ишемия нижних конечностей (Инфраингвинальное поражение): монография. Тверь: Изд. центр Твер. гос. ун-та, 225 с.

Национальные рекомендации по диагностике и лечению заболеваний артерий нижних конечностей (2019) *Ангиология и сосудистая хирургия (приложение),* **25(2):** 110 с.

Ховалкин В.Г., Тарабрин А.С., Желтов Н.Ю. и др. (2019) Отдаленные результаты эндоваскулярных вмешательств у пациентов с окклюзией подколенной артерии, страдающих критической ишемией нижних конечностей. *Ангиология и сосудистая хирургия (приложение)*, **25(2):** 468-469.

Зусманович Ф.Н. (1999) Реваскуляризирующая остеотрепанация в лечении хронической критической ишемии нижних конечностей. *Хирургия*, **№4:** 10-12.

Косаев Дж.В. (2011) Внутрикостномозговое лазерное облучение в комплексном хирургическом лечении больных с критической ишемией нижних конечностей. *Центрально Азиатский медицинский журнал*, **№4:** 213-216.

Косаев Дж.В. (2012а) Реваскуляризирующая остеотрепанация и лазерное облучение в комплексном хирургическом лечении больных с критической ишемией нижних конечностей// Вестник хирургии Казахстана, 2012. -№1.- с.46-49.

Косаев Дж.В. (2012б) Непрямые методы реваскуляризации и лазерное облучение в лечении больных с облитерирующими заболеваниями артерий нижних конечностей. *Вестник хирургии Казахстана*, **№2:** 7-9.

Кротовский Г.С., Зудин А.М. (2005) Тактика лечения пациентов с критической ишемией нижних конечностей. М.: -160 с.

Суховатых Б.С., Орлова А.Ю. (2013) Лечение критической ишемии нижних конечностей способами непрямой реваскуляризации (монография). Курск: Изд. КГМУ, 208 с.

Юнкеров В.И., Григорьев С.Г., Резванцев М.В. (2011) Математико-статистическая обработка данных медицинских исследований. Санкт-Петербург: ВмедА, 318 с.

Aslyaev A.L., Kushnaryova E.A., Stepanenko R.N., Kushnaryov A.A. (2016) Indirect revascularization occlusion of lomer limb arteries. Bulletein of Problems of Biology and Medicine, **2(part 1(128)):** 15-18.

Biancari F., Kantonen I., Albacr A. et al. (2007) Risk-scoring method for prediction of 30-day postoperative ourcomt after infrainguinal surgical revascularization for critical lower-limb ischemia: a Finnvasc registry study. *World J. Surg.*, **31(1):** 217-227.

Bosch J.L., Hunink M.G. (2007) Meta-analysis of the results of percutaneous transluminal angioplasty and stent placement for aortoiliac occlusive disease. *Radiology*, **204(1):** 87-96

Clair D.G., Dayal R., Faries P.L. et al. (2005) Tibial angioplasty as an alternative strategy in patients with limb-threatening ischemia. *Ann. Vasc. Surg.*, **19(1):** 63-68.

Dey P. (2018). Basic and Advanced laboratory techniques in histopathology and cytology. Singapore: Springer, 275 p.

Nowak-Sliwinska P., Alitalo K., Allen E., Anisimov A. et al. (2018) Consensus guidelines for the use and interpretation of angiogenesis assays. A*ngiogenesis*, **21(3):** 425-532.

Aşağı ətrafların arteriyalarının distal okklüziyaları mənşəli kritik işemiyası olan xəstələrdə dolayı revaskulyarizasiya zamanı perioperasion terapiyanın müxtəlif sxemlərində regionar qan axınının və yumşaq toxumaların mikrosirkulyasiya şəbəkəsinin vəziyyəti

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Tədqiqatın məqsədi arteriyaların distal steno-okklüziyası fonunda aşağı ətrafların kritik işemiyası olan xəstələrdə regionar qan axınının və yumşaq toxumaların mikrosirkulyasiya şəbəkəsinin vəziyyyətini dolayı revaskulyarizasiya əməliyyatları zamanı perioperasion dövrdəki müxtəlif müalicə sxemləri şəraitində öyrənməkdən ibarət olmuşdur . Tədqiqat kontingenti 2002-2019-cu illərdə müalicə almış 2 ay – 4 il müddətində kritik işemiyalı 28-74 yaşlı 210 xəstədən ibarətdir (154 kişi və 56 qadın). Kritik işemiyanın bilavasitə səbəbi - bud-dizaltı-baldır və baldır-ayaq pəncəsı seqmentləri arteriyalarının aterosklerozla və trombangitlə şərtlənmiş okklüziyasıdır. Regionar arterial və venoz qan axını göstəriciləri, dərinin oksigenlə saturasiyası qeyri-invaziv instrumental ((reovazoqrafiya,ultrasəs doppleroqrafiya , ultrasəs dupleks sonoqrafiya və s.), 80 xəstədə (55 kişi, 25 qadın) neoangiogenez və mikrosirkulyasiya şəbəkəsinin vəziyyəti isə - kritik işemiyalı aşağı ətrafların yumşaq troxumaları tru-cut bioptatlarının kompleks morfoloji-immunhistokimyəvi analizləri ilə öyrənilmişdir. Kəmiyyət göstəriciləri qeyri-parametrik statistika üsulları ilə təhlil edilmişdir. Müəyyən edilmişdir ki, sümükiliyinin lazer şüalanması ilə birlikdə aparılan revaskulyarizasiyaedici osteotrepanasiya əməliyyatı zamanı perioperasion dövrdə lazerlə venadaxili şüalandırma və sitokinoterapiya aşağı ətrafların kritik işemiyaya məruz qalmış yumşaq toxumalarında neoangiogenezi sürətləndirir, mənfəzləri sərbəst (funksional-fəal) mikrodamarların sıxlığını artırır, arterial və venoz qan axınlarının əsas göstəricilərini, həmçinin dərinin oksigenlə saturasiyası səviyyəsini, müqayisə qrupu ilə nisbətdə, əhəmiyyətli dərəcədə - bəzən isə statistik-etibarlı xarakterdə - yaxşılaşdırır. Qeyd edilən prose-

durların birlikdə aparılması əməliyyatdan və müalicədən sonrakı bir neçə illik dövrdə də xəstələrin klinik vəziyyətinin və öyrənilmiş göstəricilərin nisbi-qənaətbəxşliyinə şərait yaradır.

Açar sözlər: *Arteriyaların okklüziyası, kritik işemiya, dolayı revaskulyarizasiya, lazer şüalanması, sitokinoterapiya, regionar hemodinamika, mikrosirkulyasiya*

Состояние регионарного кровотока и микроциркуляторного русла мягких тканей при различных схемах периоперационной терапии при непрямой реваскуляризации у больных с критической ишемией, вызванной окклюзией дистальных артерий нижних конечностей

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Целью настоящего исследования являлось изучение состояния регионарного кровотока и микроциркуляторной сети мягких тканей у больных критической ишемии на фоне дистальной окклюзии артерий нижних конечностей в зависимости от лечебной тактики в периоперационном периоде при непрямой реваскуляризации. Исследование проводилось у 210 больных (154 мужчин, 56 женщин) с критической ишемией нижних конечностей в возрасте от 28 до 74 лет, находящихся в стационарном лечении в 2001-2019 гг., продолжительностью ишемии в течении от 2-х мес. до 4-х лет. Причиной критической ишемии явилась окклюзия артерий бедренно-подколенно-берцового и берцово-стопного сегментов атеросклеротического и тромбангиитического генеза. Неинвазивными инструментальными исследованиями (реовазография, ультразвуковая допплерография, ультразвуковое дупплексное сканирование и др.) были изучены показатели регионарного кровотка. В tru-cut биоптатах мягких тканей ишемизированной конечности у 80 больных (55 мужчин, 25 женщин) комплексными морфологическими и иммуногистохимическими исследованиями же было изучено состояние микроциркуляторного русла и неоангиогенеза. Цифровые показатели были обработаны статистическими методами для непараметрических критериев. Выявлено, что применение внутривенного лазерного облучения крови и цитокинотерапии ронколейкином в периоперационном периоде при реваскуляризующей остеотрепанации с внутрикостномозговым лазерным облучением, по сравнению с контрольной группой, ускоряет неоангиогенез, увеличивает число функционально-активных микрососудов со свободным просветом в мягких тканях ишемизированной конечности. В сравнении с контрольной группой, непрямые методы реваскуляризации статистически достоверно улучшают показатели регионарного кровотока и сатурацию кожи кислородом. Сочетанное применение внутривенного лазерного облучения крови и цитокинотерапии в периоперационном периоде при непрямой реваскуляризации остеотрепанацией с костномозговым лазерным облучением создает благоприятные условия для стимуляции регионарного кровотока и активации неоангиогенеза с улучшением микроциркуляторного русла в критически-ишемизированных нижних конечностях.

Ключевые слова: *Окклюзия артерий, критическая ишемия, непрямая реваскуляризация, лазерное облучение, цитокинотерапия, регионарная гемодинамика, микроциркуляторное русло*